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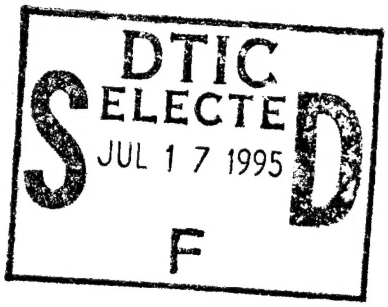

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Live Fire Support Services

Final Report

Period Covering 15 November 1993 - 31 December 1994
Contract No. DAMD17-94-C-4008

Prepared for:

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The N-gas model is an empirical tool developed by the fire science community to predict toxic potency of various materials when burned based on the chemical nature of gases produced. This research is extending applicability of the N-gas equation to military scenarios by expanding the empirical formula to include the effects of exposure to NO2 (in rodents). Rats are exposed, nose-only and under controlled conditions, to nitrogen dioxide, singly and in combination with carbon monoxide, carbon dioxide and air. LC50 values are determined based on 30 minute exposure, followed by 2 week post exposure. Blood gas, hemoglobin and respiratory data are collected.

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FOREWARD

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Date 10 Jan 95

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Live Fire Support Services:

Expansion of the N-Gas Model for Toxic Potency to Include NO_2 Effects

FINAL REPORT

Background

The survivability of armored combat vehicles, ACVs, depends on the vulnerability of both the vehicle and the crew. Until a few years ago, evaluation of the vehicle vulnerability was limited to an assessment of the armor's ability to prevent penetration by a specified anti-armor threat and an evaluation of the vehicle systems and components through selective engineering tests. However, questions arose regarding the accuracy of assessing weapon platforms through selective component testing and then extrapolating by computer modeling to determine ACV vulnerability/survivability in combat. This prompted the Office of the Secretary of Defense to initiate the Joint Live Fire Program in 1984. Congress then passed Live Fire Test legislation in 1987 to require live fire testing of all United States weapon platforms against realistic combat threats (Amendment Title 10, U.S.C. 139). This law stipulated a weapons platform may not proceed beyond low-rate initial production until "realistic survivability testing is complete". The purpose of such testing is fourfold:

a. To assess the vulnerability of U.S. weapons systems to realistic combat threats.
b. To assess the lethality of U.S. conventional combat systems against foreign weapons systems.
c. To produce design changes which would increase crew and/or vehicle survivability on the battlefield.
d. To produce a database which would be used to improve computer modeling of weapons systems vulnerability.

"Behind-armor" events produce a number of potential hazards to the crew including fragment injury, blast overpressure, and exposure to toxic gases produced by burning materials or the penetrating object. Improvements in design and protective gear have greatly enhanced the survivability of the crew in recent years. The crew in a present day ACV incident is more likely to survive, to remain in the vehicle and to continue the combat mission. This likelihood increases the importance of accurately evaluating the ancillary effects of such penetrations as possible causes of crew injury or incapacitation. It also raises the question of possible health risks created within the vehicle should the crew remain inside after armor penetration. For these reasons, the Army Medical Department was tasked to evaluate nonfragment injuries for live fire testing. This action has required researchers to identify potential hazards and to standardize instrumentation to collect data representative of injurious circumstances.

Criteria have been established capable of predicting injury due to blast overpressure, thermal exposure, accelerative loads and toxic gases. Unfortunately, medical literature has revealed little data which can be applied directly to these environments. For example, in the area of toxic gases, most medical studies reported in the literature focus on low level chronic exposures typical of environmental exposures to air pollutants. In order to confidently assess injury potential under battlefield conditions requires investigation through medical research specifically designed to characterize the hazards in these environments. Predicting injuries and fractional incapacitation in these environments is not an exact science and much work remains to be done.

The Walter Reed Army Institute of Research Department of Respiratory Research has been tasked with development of these criteria and models. Previous toxic gas research funded and/or conducted by Department of Respiratory Research personnel has focused on brief exposures to high levels of nitrogen dioxide, probably the least characterized of the potential toxic combustion gases found in battlefield scenarios. Additional research into the sublethal effects of combinations of gases, such as those in an explosion or blast, was needed.

Two groups of researchers (outside DoD) have looked at the lethal or incapacitating effects of combustion gases - the Federal Aviation Administration (FAA) and the Fire Safety Department at the National Institute of Standards & Technology (NIST), formerly the National Bureau of Standards. The FAA has evaluated the atmospheres generated in typical fires on board aircraft to determine minimum survival times for escape and maximizing the time to flashover. NIST researchers and the fire safety & commercial industries have evaluated various methods for determining the toxic potency of burning materials by looking at lethality levels in animal exposures. One method, known as the "N-gas model", developed by the Fire Research Department at NIST seemed especially promising as a starting point for the evaluation of sublethal effects of combinations of toxic combustion gases.

During the 1970's, knowledge about the toxicity of (burning) materials was considered a significant "missing link" in the evaluation and understanding of fire hazard. The need for a small-scale laboratory procedure to ascertain the toxic potency of the combustion products was highlighted by research which indicated that the combustion products from an experimental fire-retarded rigid polyurethane foam caused seizures and death in rats, while the same foam without the fire-retardant did not produce any abnormal neurological effects. The toxicity of the foam was ultimately traced to the formation of a bicyclic phosphate ester in the smoke. This result raised an alarm about the possible presence of "supertoxicants" or unexpected compounds in smoke from burning or smoldering materials. Since the presence of this bicyclic phosphate ester would not have been detected by ordinary chemical analysis of the smoke, this research also emphasized the need for animals as measurement "instruments". The need for a combined biological and chemical approach was obvious. The observation of adverse effects in rodents would indicate the presence of unusual toxicants or

synergistic effects of combined toxicants which might not be discovered by routine chemical analysis.

A number of organizations, including NIST, embarked on extensive research programs to develop standardized methods to assess and predict the toxic "potency" of various materials under controlled combustion conditions.

Despite differences in approach, all of these methodologies had the following elements in common: laboratory animals were exposed under controlled conditions to smoke & combustion by-products atmospheres produced by the burning of various materials under prescribed conditions, including control of temperature, time of exposure, air temperature, availability of oxygen, combustion temperature, air movement and air flow. Most fire protection/prevention researchers used LC_{50} as the endpoint although some work has been done using incapacitation levels. The results were normally reported as the mass of the substance (under test) which was consumed to produce the effect.

Concurrent with developing an accepted standardized test methodolgy for assessing toxic potency of combustion by-products, researchers at NIST also developed a mathematical model or formula to "predict" the toxic potency of mixtures of these gases. This model, known as the "N-Gas Model", was based on the premise that a small number ("N") of gases in smoke account for the majority of observed toxic potency. The lethality of each of these gases was determined for laboratory animals using pure gases rather than actual combustion atmospheres; similar measurements for combinations of these gases indicated whether the gases were additive, synergistic, or antagonistic.

NOTE: Additive effects are defined as the case in which the predicted (and observed) response due to a combination of agents is equal to the sum of responses observed when both agents are administered separately. Toxicological interactions are considered to be synergistic when the response to combined agent exposure exceeds the sum of responses when each agent is administered separately. Antagonistic responses occur when the response to combined agent exposure is less than the sum of responses to individual agent exposures. Additionally, there are two distinct types of additive responses: "dose additive" in which two or more agents target the same receptor with similar efficiencies, but not necessarily the same potencies or "effect additive" in which agents act on different targets to produce similar effects.

The results of these mixed gas studies were reduced to an algebraic equation which has been empirically determined for the exposure of rats to mixtures of CO_2 (carbon dioxide), CO (carbon monoxide), HCN (hydrogen cyanide), reduced O_2 (oxygen) and HCl (hydrogen chloride).

The concept that simple additivity may be sufficent to explain the toxicity of mixtures of fire gases has been investigated since the late 1960's and in the mid 1970's, Gordon Hartzell of Southwest Institute of Research in San Antonio, TX was proposed the term

"Fractional Effective Dose" or FED to name the variable which quantifies what fraction of a lethal dose the animal has received.

Since the actual dose delivered to an animal via inhalation cannot normally be quantitatively determined, researchers modified the FED to consider instead an exposure dose, defined as the product of the gas concentration in the atmosphere multipied by the time of exposure. For the case of simple additivity of effects, the FED is simply

$$FED = \sum (C_i \, dt) / LCt_{50} \, (i)$$

where Ci is the concentration of the "i"th gas species and LCt50 (i) is the [lethal concentration] X [time] product for the gas species. Experimental work conducted by NIST (and others) has borne out that mixtures of important toxic gases follow this relationship, with modification.

The current N-gas model, as developed by NIST researchers, includes six gases: reduced oxygen, carbon dioxide, carbon monoxide, hydrogen bromide, hydrogen chloride and hydrogen cyanide. The most current form of the N-gas model as developed by NIST researchers is as follows

Equation 1:

$$FED = m[CO]/[CO2\text{-}b] + [HCN]/LC50(HCN) + (21\text{-}[O2])/15.6 + [HCl]/LC50(HCl) + [HBr]/LC50(HBr)$$

where

FED = A calculated number representing the concentrations & interactions of the various gases in the exposure atmosphere).

m,b = Empirical constants derived from experimental curves, and incorporating the "synergistic" effects of CO_2 concentration on the LC_{50} of CO.

[gas] = The concentration of a given gas in the exposure atmosphere given in ppm for all gases except O_2 which is expressed in volume %.

LC_{50}(gas) = Concentration of a given gas lethal to 50% of the rats exposed for 30 minutes in a static environment, i.e. the LC_{50} value. These concentrations are expressed in ppm for everything except O_2 which is expressed in volume %

The first term reflects the potentiation of CO by the presence of CO_2. Studies at NIST demonstrated that although CO_2 has a very low toxicity of itself (on rats), its effect on mixtures is not as slight as linear additivity would suggest. As the concentration of CO_2 increases, the (apparent) toxicity of CO increases; above 5%, the toxicity of CO begins to decrease again. The values for m and b in the above equation represent the constants derived

in a simple regression equation and are "m" = (-18) and "b" = 122000 for CO_2 concentrations of 5% or less and "m" = 23 and "b" = (-38600) when CO_2% exceeds 5%. Carbon dioxide also increases the toxicity of other gases currently included in the model as well as that of NO_2. However, for simplicity, the effect of the CO_2 was added into this equation only once. Since CO is generally the dominant toxicant in nearly all real fires, the CO_2 effect was merged into the CO factor (only).

The LC_{50} values for 30-minute exposures for the other linear terms in the equation are as follows: HCN, 150 ppm; HCl, 3800 ppm; and HBr, 3000 ppm.

The third term in the equation arose because oxygen itself is not a toxicant, instead, its lack is what is toxic. Thus, the form for O_2 is (21-O_2). The 30 minute LC_{50} of O_2 is 5.4% which is subtracted from the normal concentration of O_2 in air, 21%.

Even with these non-linearities, the N-gas model equation still exhibited some systemic deviation from the ideal: 50% of the animals should die at an FED of 1.0; instead, the 50% lethality level corresponds to an FED of 1.1. Nonetheless, NIST researchers considered the model well enough established to be offered for engineering use, cautioning that nitrogen oxides, especially NO_2, needed consideration and that the antagonistic effects of NO_2 and HCN required further study.

The current N-gas model, as developed by NIST researchers, includes seven gases: reduced oxygen, carbon dioxide, carbon monoxide, hydrogen bromide, hydrogen chloride, hydrogen cyanide and hydrogen fluoride. The primary focus of the current study was to evaluate the effects of NO2 on the combined LC50 values of the N-gas model and to revise the model to include it.

Other researchers, especially in studying the effects of toxic combustion gases on humans, describe variations of the N-gas model in which the various gases are grouped together, based on their toxicological action, and then combined. David Purser, director of the Huntingdon Research Centre in England, describes a well developed model, incorporating gases which are narcotic in action with the respiratory enhancement of elevated levels of carbon dioxide which are often found in fires. His model does not include nitrogen dioxide or any other irritant gas. Dr. Lousie Spietel at the FAA Technical Center in Atlantic City, has developed a model which combines the intoxication activity of carbon monoxide with the lethality effects of the irritant gases to determine maximum escape time for passengers to exit the aircraft before being incapacitated and assess hazard levels in aircraft cabin fires. This model is strictly a mathematical model which was based on data from the literature of actual animal testing done by other researchers.

Anticipating future research needs, the contractor not only collected data on the gas concentrations in the animal exposure chamber and noted lethality results, but also collected the following additional data parameters: arterial and mixed blood gases, hemoglobin, methemoglobin, carboxyhemobloin, respiratory rates, tidal volumes, minute volumes, post

exposure growth curves, and lung weights. This data is included as Appendices to this report and/or is available electronically at WRAIR.

Preliminary analysis of the data indicates that interactions defined as "antagonistic" or "synergistic" by Levin in developing the N-gas model can be related to the minute volumes and respiration rates. If this relationship can be further validated and successfully demonstrated in follow-up studies and additional data analysis (outside the scope of the present contract), then the development of mathematical formulas or models to predict the sublethal (respiratory) effects of such exposures would be facilitated.

Methodology

Mature male Sprague Dawley rats weighing 250 - 350 grams (at time of exposure) were procured from Charles River Laboratory, Boston MA, and placed in quarantine in accordance with WRAIR SOPs. After delivery from quarantine, animals were randomized, segregated into groups of eight animals per group, identified by eartags and allowed to acclimate for one week prior to exposure housed in individual micro-isolators. Animals were weighed at least every two days and monitored for normal growth.

On the day before the planned exposure, two animals were selected from the group for catheter placement. The procedure used is described in detail in Appendix A. The catheter (a six inch length of PE50 tubing) was placed in the caudal tail artery and sutured in placed. The animal was placed in a jacket collar which allowed movement but prevented access to the tail area and returned to the cage for the night.

The exposure chamber consisted of a 134 liter Plexiglas box, about 4 feet long, 12 inches wide and 16 inches high. Reagent grade gases were combined at atmospheric pressure in a four-channel stainless steel mixer to the preselected composition and delivered to the inlet port on the chamber (Figure 1) via 0.250"ID Teflon tubing.

The composition of the atmosphere inside the chamber was constantly monitored by gas analyzers. Individual pumps on the analyzers drew air samples through a six-port sampling manifold made of nonreactive plastics and into the appropriate analyzer cell. After analysis, the air was returned to the chamber via return ports on the top of the chamber. Excess air flow in the sampling manifold was returned to the chamber via a return inlet on the bottom of the chamber. This arrangement maintained constant mixing while simulating the closed atmospheres typical of an explosion/fire in an armored personnel carrier or tank.

The following gases [in the exposure chamber atmosphere] were monitored: % oxygen by electrochemical analyzer (Ametek model S3A-1, Thermox Instruments, Pittsburg, PA); % carbon dioxide by infrared analyzer (Ametek model CD-3A, Thermox Instruments, Pittsburg, PA); carbon monoxide, ppm levels, by nondispersive infrared-ultraviolet (IR-UV) spectrophotometer, (Binos, Inficon Leybold-Heraeus, Federal Republic of Germany); and nitrogen dioxide, ppm levels, by dual-beam infrared-ultraviolet (IR-UV) spectrophotometer

(Binos, Inficon Leybold-Heraeus, Inc., Federal Republic of Germany). Atmposheres were also sampled for contamination by nitrogen monoxide (NO) using dual-beam infrared-ultraviolet (IR-UV) spectrophotometer (Binos, Inficon Leybold-Heraeus, Inc., Federal Republic of Germany). NO was not detected as a contaminate in any of the exposures.

The exposure chamber contained an 8-liter inner chamber which was gasketed from the exposure atmosphere. This inner box contained the inlet portholes into which the animal restrainers were placed. Medical grade breathing air was pumped through this chamber while the animals were being loading into the chamber prior to exposure. The chamber was fitted with a remotely operated door which was released, dropping to the floor of the chamber and allowing the test atmosphere to contact the animals. The thirty minute exposure time started at the moment the door was dropped. The test atmosphere as monitored by the various analyzers equilibrated in less than 90 seconds.

The animals to be exposed were placed into Lexan restrainers [Part number 70054 sleeve fitted with part number 70057stainless steel tail tube/back plate, Lab Products, Inc., Rockville, MD]. These restrainers are designed for use in flow-through nose-only exposure chamber. The animal was placed in the tube and his tail was fed into the tail tube; the metal tail tube acted as a heat sink for the tail to moderate increases in body temperature during the prolonged restraint required for these inhalation exposure studies. The back plate was then adjusted snuggly against the animal's rump, positioning the nose firmly into the inlet port. The animals were placed into the restrainers as quickly as possible to minimize stress and loaded into the exposure chamber at once. As noted above, the animals were exposed only to medical grade air, flowing at 12 to 18 cubic feet per minute, until the exposure door was opened.

Eight animals were exposed in each test. No animal was exposed more than once. Additional sets of animals were run as controls, breathing only medical grade air or medical grade air containing 5% carbon dioxide. As noted above, two of the animals had been fitted the previous day with catheters in the caudal tail artery. Those two animals were placed in tube restrainers which had been modified to allow access to the tail. Pre-exposure (or "zero time") blood samples were drawn from each animal just prior to being loading into the chamber for analysis. Additional samples were drawn at 5 minutes into the exposure, 10 minutes into the exposure, 15 minutes into the exposure, at 30 minutes (the end of the exposure), at 30 minutes post-exposure and at 90 minutes post exposure. Samples were drawn alternately from the two catheterized animals so that one animal was sampled at 0, 5, 15 and 30 minutes post, while the other was sampled at 0, 10, 30 and 90 minutes post. Marquest part number 601 "Aspirator" blood gas syringes containing 50 units of dry lithium heparin were used for all blood samples [Marquest Medical Products, Englewood, CO]. At least 0.7 mls of blood was drawn to minimize concentration effects from the heparin.

All samples were analyzed as quickly as possible for pH & blood gases (Instrumentation Laboratories IL1306 analyzer) and hemoglobin levels (Radiometer-Copenhagen OSM3 Hemoximeter).

In addition, three of the remaining six animals were selected at random and placed in restrainers which had been modified with adaptors designed to receive a linear pneumotach [Model 8411B non-heated pneumotachometer, Hans Rudolph, Kansas City, MO]. The modification of the restrainer allowed it to be used as a partial body phythesmography enclosure - the deflections of the chest wall during respiration were recorded as changes in the pressure across the screens in the pneumotach housing. The inlet and outlet ports on the pneumotachs were connected to low range differential pressure transducers [MP45-20-817, Validyne Engineering Corporation, Northridge, CA]. Pressure flow data was collected at the rate of 40 samples/second/channel and recorded using a computerized data acquisition system [DATAQ Instruments, Akron, OH]. Complete respiratory curves were recorded for the three animals throughout the thirty minutes exposure duration. Respiration rate and time of death were obtained from these recordings. The curves were integrated and compressed to yield inspired minute volume, expired minute volume, tidal volume, and delta [V], the difference in inspired and expired volume.

Lung weights were done on selected animals, both in controls and in exposures involving NO2, either alone in air or combined with carbon monoxide. Typically, increases in the ratio of "wet" lung weight to dry lung wieght is a qualitative indicator of pulmonary edema or damage from irritant inhalants. Immediately after euthanasia, the chest cavity of the catheterized animals was opened and the lungs removed. The lobes were separated, perfused with saline, blotted on paper toweling and placed in pretared petri dishes. The lungs were weighed and then placed into an oven at 36C and reweighed every 12 hours to constant weight.

At the conclusion of the thirty minute exposure time, all eight animals were quickly removed from the chamber and returned to normal air. The non-catheterized animals were removed from the restrainers, visually assessed for physical condition and returned to individual cages in the laboratory. The animals were closely monitored for two hours following exposures as it was noted that deaths typically occured within or shortly after exposure. No deaths in any of the exposures occured more than 24 hours post exposure (unlike observations reported by NIST researchers).

At the end of the two hour post-exposure observation period, the animals were returned to their microisolator cages in the animal room. Body weights were recorded daily for at least 14 days following the exposures; in some cases, wights were tracked for 30 or 45 days.

After final blood samples were collected from the two catheterized animals, the animals were lightly anethesized with Ketamine/Rompun (0.1 ml per 100 g) and euthanized in a CO2 chamber. After euthanasia, the lungs were removed, if appropriate to the exposure protocol.

During exposures involving NO2, the lungs of any animals which died during or post-exposure were also removed for wet/dry lung weight analysis.

Results/Discussion

Table I summarizes the exposures conducted and the lethality results. These combinations were selected to validate LC50 values reported by other researchers and in prior WRAIR-supported NO2 work. It is recognized that simple binomial expansion probablility suggests that the results obtained are not statistically valid, but, since the intent was merely to double-check previously reported LC50 values in adult male rats, duplicate exposures were not conducted.

Table II details the N-gas values or "FED" values expected from the various exposure concentrations. The column entitled "NIST value" gives the N-gas value using the existing equation (Equation 1). The column entitled "Levin N-gas value" lists the N-gas value obtained using Levin's newly revised N-gas model equation [Levin, B.C., "Further Development of the N-Gas Model: Effects of NO2 Induced Injury", unpublished data submitted to WRAIR December 14, 1994] which incorporates NIST results of multigas exposures including NO2. (Equation 2) The column labeled "N-gas extension" reports theFED or N-gas value if the simplistic approach of simply adding NO2 as another linear term, similar to the hydrogen halides. (Equation 3)

Equation 1:

FED = m[CO]/[CO2-b] + [HCN]/LC50(HCN) + (21-[O2])/15.6 + [HCl]/LC50(HCl) + [HBr]/LC50(HBr)

Equation 2:

FED = m[CO]/[CO2-b] + {[HCN]/LC50(HCN) X (0.4)[NO2]/LC50NO2}+ (21-[O2])/15.6 + [HCl]/LC50(HCl) + [HBr]/LC50(HBr) + (0.4){[NO2]/LC50(NO2)}

Equation 3:

FED = m[CO]/[CO2-b] + [HCN]/LC50(HCN) + (21-[O2])/15.6 + [HCl]/LC50(HCl) + [HBr]/LC50(HBr) + [NO2]/LC50(NO2)

where

FED = A calculated number representing the concentrations & interactions of the various gases in the exposure atmosphere).

m,b = Empirical constants derived from experimental curves, and incorporating the "synergistic" effects of CO_2 concentration on the LC_{50} of CO.

TABLE 1

EXPOSURE SCHEDULE SUMMARY

EXPOSURE CONDITIONS & RESULTS			AVERAGE GAS CONCENTRATIONS			
Test date	Exposure Conditions	Results	O2%	CO2 ppm	NO2 ppm	CO ppm
25-Aug	AIR CONTROLS	0/6 DIED	20.4	5100	0	0
30-Aug	5% CO2 CONTROLS	0/6 DIED	19.5	52200	0	0
1-Sep	2000 CO IN AIR	0/6 DIED	19.5	12800	0	2010
7-Sep	4500 CO IN AIR	4/6 DIED	19.9	8300	0	4500
9-Sep	2500 CO IN 5% CO2	1/6 DIED	18.84	56000	0	2340
16-Sep	3500 CO IN 3.5% CO2	3/6 DIED	19.3	36000	0	3300
21-Sep	150 NO2 IN AIR	0/6 DIED	19.5	8700	140	0
28-Sep	200 NO2 IN AIR	3/6 DIED	19.4	9500	187	0
30-Sep	120 PPM NO2 IN 5% CO2	2/6 DIED	18.6	50830	109	0
4-Oct	3500 CO & 110 NO2 IN AIR	6/6 DIED	19.66	7484	114	3550
7-Oct	2080 CO & 95 NO2 IN AIR	1/6 DIED	19.38	10570	104	2090
14-Oct	2630 CO & 111 NO2 IN AIR	5/6 DIED	19.43	11400	111	2632
18-Oct	1940 CO & 107 NO2 IN 4.5% CO	2/6 DIED	18.83	45113	107	1940

TABLE 2

N-Gas Values Using Different Equations

Exposure Conditions	Results	NIST N-gas	Levin N-gas	Ext'd N-gas
AIR CONTROLS	0/6 DIED	0.04	0.04	0.04
5% CO2 CONTROLS	0/6 DIED	0.1	0.1	0.1
2000 CO IN AIR	0/6 DIED	0.43	0.43	0.43
4500 CO IN AIR	4/6 DIED	0.78	0.78	0.78
2500 CO IN 5% CO2	1/6 DIED	0.71	0.71	0.71
3500 CO IN 3.5% CO2	3/6 DIED	0.8	0.8	0.8
150 NO2 IN AIR	0/6 DIED	0.1	0.38	0.8
200 NO2 IN AIR	3/6 DIED	0.1	0.48	1.04
120 PPM NO2 IN 5% CO2	2/6 DIED	0.15	0.37	0.7
3500 CO & 110 NO2 IN AIR	6/6 DIED	0.64	0.87	1.21
2080 CO & 95 NO2 IN AIR	1/6 DIED	0.44	0.65	0.96
2630 CO & 111 NO2 IN AIR	5/6 DIED	0.53	0.75	1.08
1940 CO & 107 NO2 IN 4.5% CO2	2/6 DIED	0.59	0.81	1.13

[gas] = the concentration of a given gas in the exposure atmosphere given in ppm for all gases except O_2 which is expressed in volume %.

LC_{50}(gas)= concentration of a given gas lethal to 50% of the rats exposed for 30 minutes a static environment, i.e. the LC_{50} value. These concentrations are expressed in ppm for everything except O_2 which is expressed in volume %

As can be seen from comparing the predicted FED values with the lethality rates from the exposures, it is apparent that the N-gas model (in any version) has some limitations. For example, for exposure atmospheres in which the CO/CO2 ratio is artificially manipulated and is at levels not normally found in building fires, the N-gas value provides only a mediocre prediction of actual lethality results. Additionally, for atmospheres containing NO2 but not HCN, the N-gas value predicts a much lower lethality rate than is actually observed.

The N-gas value and the N-gas equation appear to have limited application to the prediction of lethality under actual conditions. The usefulness of the model to predict lethality based on toxic gas measurements made during Live Fire tests is also questionable. The N-gas equation has been developed to predict the toxic potency of various materials when combusted under rigorous & controlled laboratory conditions, using a carefully defined animal model. Based on the results of these experiments and those of other researchers in the field, reliable prediction of toxic gas effects, especially sublethal effects under actual battlefield conditions, will require a much more sophisticated model, incorporating such variables as respiratory rates, exercise levels, temperature, inspired volumes, and others. A predictive model based only on average gas concentrations over a defined time interval is simply not adequate.

Other Objectives

The "Live Fire Support Services, Expansion of the N-Gas Model for Toxic Potency to Include NO2" contract was a one-year duration support services contract; contract objectives to be accomplished included the following items:

1. Assess the possiblities of applying the N-gas model approach to evaluating the "toxic potency" of various materials of military significance, considering that the existing model was developed to assess toxic potency of common commercial & residential construction and furnishings materials.
2. Analyze the existing N-gas model protocol for changes and enhancements which would better represent the situations existing in a military situation or combat fire scenario.
3. Utilizing unpublished data provided by researchers at NIST, revise the existing N-gas model to add NO2.

4. Establish a small-animal exposure facility at the WRAIR Department of Respiratory Research laboratories, using equipment transferred from NIST researchers.
5. Validate the N-gas model as revised to include the effects of NO2 using rodents.
6. Provide support as necessary to Department of Respiratory Research investigators in validation of the revised N-gas model in a large animal protocol.

Results supporting the stated objectives are addressed below.

Objective 1:

Assess the possiblities of applying the N-gas model approach to evaluating the "toxic potency" of various materials of military significance.

Summary: The N-gas model procedure has been researched for applicability to the determination of toxic potency of materials of military significance. The procedure for animal exposures to combustion by-products is suitable to these determinations, however, there are accepted alternate procedures which do not utilize animals and are more appropriate for routine screening purposes. The real strength of the N-gas model methodology is the potential to develop it into a mathematical model for the prediction of toxic effects from mixtures of combustion gases.

The N-gas model protocol in its current form has been validated against a large-scale combustion scenario for a few typical residential construction and furnishing materials. As a result of this study, it was determined that the N-gas model combustion exposure protocol does not produce the same combustion atmosphere as the full-scale test but, given knowledge of the combustion atmosphere in an enclosure, the N-gas model formula can provide accurate predictions of the occurrence of animal deaths.

Since the N-gas model uses animals in the exposure phase, there is little chance that unknown toxicants will go undetected. Identification of the unknown toxicants remains an involved & technological challenge, but identification is not a critical requirement of a screening procedure.

The commercial aviation community and the Federal Aviation Administration have developed a number of acceptable screening tests for toxicity. These include Airbus Industries procedure ATS1000.01, Boeing Aerospace procedure BSS 7239, British Aerospace BAEP 4623, and Douglas Aircraft DMS 2294.

An extensive literature search was conducted to survey the current state of toxic potency testing. Results of all literature searches and copies of selected reports are already on file in the Department of Respiratory Research at WRAIR.

Objective 2:

Analyze the *existing* N-gas model protocol for changes and enhancements which would better represent the situations existing in a military situation or combat fire scenario.

Summary: Literature review suggested that the N-gas model methodology was a candidate methodology for application to military scenarios.

The N-gas model (as it currently exists) is based on thirty minute constant level exposures at normal room temperature and under static conditions (ie without significant airflow) After analyzing typical conditions reported during Live Fire testing conducted by the Live Fire Directorate at Aberdeen Proving Grounds, the following changes and enhancements to the N-gas model exposure conditions were explored.

a) Shortening the exposure interval - thirty minutes is unrealistically long for conditions to remain static. Two, five and ten minute exposures are more representative of conditions encountered in an actual combat scenario. Air controls and NO2 in air exposures were conducted for 15 minutes rather than 30 to assess differences. Although N-gas model values cannot be evaluated without extensive testing to establish LC50 values for 15 minute exposures, respiratory values were recorded. Those data curves are included in the Appendices.

b) Adding a term (into the equation) to account for temperature effects thermal gradients in the Live Fire test environment can be significant and would certainly contribute to survivability, given the same concentrations of toxic gases. Not only does increasing temperature drive the kinetics of the chemical and biochemical interactions, it also causes detrimental effects of its own. The Federal Aviation Administration (FAA) has developed empirical formulas to gauge the effects of elevated temperature on incapacitance. The incorporation of a modified thermal gradient factor needs to be investigated in follow-on studies.

c) Increasing gas concentration levels - researchers at Aberdeen Proving Grounds have reported levels of NO2 exceeding 10,000 ppm. Although such high concentrations disperse very quickly, refinement of the N-gas model and procedure to look at very high exposures coupled with shortened time intervals would allow more accurate representation of actual field conditions. Since LC50 values at 30 minute exposures were the toxicological endpoint of this study, no concentration levels approaching LC100 were studied.

Objective 3:

Revising the existing N-gas model to incorporate unpublished data to be provided by researchers at NIST.

Summary: The data required to accomplish this revision was not furnished. A seperate report ["Further Development of the N-Gas Model: Effects of NO2 Induced Injury", Contract MIPR 41720023, by B.C. Levin, dated 13 December 1994, summarizing the results of testing done by researchers are NIST] was submitted to WRAIR Department of Respiratory Research after the contract term for this project had expired.

Objective 4:

Establishment of a small-animal exposure facility at WRAIR Department of Respiratory Research laboratories, using equipment transferred from NIST researchers.

Summary: Several of the instruments needed to set-up the gas analysis and distribution systems were not included in transferred inventory. Other equipment items were not in operating condition. Rather than investing in expensive and time-consuming repairs or refurbishments, other government equipment available within WRAIR is being utilized where appropriate.

The small animal exposure apparatus and instrumentation described under "Methods" in this report was developed for this objective. The set-up is also capable of providing gas mixtures for support of the large animal study (see Obj. 6) and of recording respiratory data for other small animal studies being conducted by the Department.

Objective 5:

Validate the N-gas model to include the effects of NO2 in a small animal model.

Summary: Completed, see ***Results*** section of this report for discussion. See also Protocol M03-94, "Expansion of the N-Gas Model for Prediction of Toxic Potency of Combustion Gases to Include NO2 Effects in the Rat", S. M. Smith. The experimental methodology described in the protocol was based on the methodology utilized by NIST researchers in their pure gas and gas combination interaction studies.

Objective 6:

Provide support as necessary to Department of Respiratory Research investigators in validation of the revised N-gas model in a large animal protocol.

Summary: JAYCOR investigator co-authored the large animal validation protocol, approved by the LACUC in September 1994 and included in this report in the Appencies and assisted Department of Respiratory Research in the large animal validation studies.

APPENDICES

Large Animal Validation Protocol

MO 23-95

"Pathophysiologic Effects of Combined Toxic Gases/Mock Live Fire Scenario in Conscious Sheep"

PROTOCOL No. MO 23-95

TITLE: Pathophysiologic Effects of Combined Toxic Gases/Mock Live Fire Scenario in Conscious Sheep

DIVISION: Medicine

DEPARTMENT: Respiratory Research

RESPONSIBLE INVESTIGATORS: see attached

Principal Investigator:

Co-Investigators:

Department Chief: (OCONUS TDY) Asst C, ORR

Division of Medicine, Director:

Veterinary Consultant:

COORDINATION: When protocols require support from other Divisions, investigators must coordinate with the Division involved and obtain signatures.

Supporting Divisions: Pathology, Veterinary Medicine

C, Dept Ultrastructure (Path)

C, Dept Comp Pathology (Path)

C, Dept Anim Res (Vet Med)

MANAGEMENT DATA: Short Title: Fire Toxicity in Sheep

Project: STO:Y Task:C APC:WICS USDA Code:D
Beginning Date: Oct 1994 Ending Date: Dec 1994

Pathophysiologic Effects of Combined Toxic Gases/Mock Live Fire Scenario in Conscious Sheep

Saftey Officer, WRAIR

BERT J. MUECK

Principal Investigator

ADOLPH J. JANUSZKIEWICZ

Pathophysiologic Effects of Combined Toxic Gases/Mock Live Fire Scenario in Conscious Sheep

Principal Investigator: Adolph J. Januszkiewicz, Ph.D., DAC

Co-Investigators: Shiela Smith, M.S.
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Management Data

Project:
STO: Y
Task: C
APC: WICS
Work Unit#: W03KAA

BACKGROUND

Death or injury by fire continues to be a major health concern in the United States, affecting thousands of Americans yearly. Injury by fire is usually associated with intense heat and burns. However, equally or more important are the debilitating or lethal effects of fire resulting from "smoke inhalation", which can lead to cardiopulmonary derangement, asphyxia, lung edema and death.

"Smoke inhalation" is in itself a generic term. "Smoke" generally refers to suspended particles in air resulting from combustion or pyrolysis of organic materials. In reality, injury induced by "smoke inhalation" is a complex phenomenon. In a typical fire scenario, suspended particles (carbon, metal oxides, *etc*) are coincidentally inhaled with a wide variety of gases, vapors and fumes of high toxicity. The composition of fire gases varies greatly from fire to fire, depending on heat intensity, nature of the burned materials, and numerous other factors. However, some qualitative gas composition commonalities exist among different fires. For example, common combustion gases include oxides of carbon (carbon monoxide, CO, and carbon dioxide, CO_2), and oxides of nitrogen (nitrogen monoxide, NO, and nitrogen dioxide, NO_2) from incomplete combustion of carbonaceous materials, and fixation of ambient nitrogen, respectively, low oxygen (O_2), as O_2 is consumed by fire, cyanide (HCN) from the burning of materials such as nylon, wool, silk and polyurethane, and halides (HCl, HBr) from the burning of chlorinated acrylics and polyvinyl chlorides. This proposal addresses the toxicity resulting from exposure to these simple combined gases, in the absence of particulate matter and heat, in a large animal experimental model.

RATIONALE

Although the importance of fire inhalation injury has been understood, and post exposure supportive measures have been developed for some time, focus on injury mechanisms and thresholds of injury is more recent, as new technologies have allowed greater insight into the complex physio-chemical characteristics of fire. A better understanding of the primary insult will ultimately lead to newer and better avenues of treatment of inhalation injury.

Many animal studies have focused on the toxicity of individual combustion gases (CO, NO_2, halides) (Wang *et al*, 1990; Januszkiewicz *et al*, 1992). Some animal studies have addressed the toxicity of heat and particulate matter on cardiopulmonary function (Loick *et al*, 1992). Few animal studies have addressed the toxicity of combustion gases in combination. Toxicity modelling can greatly assist in the development of treatment/prophylaxis modalities, while decreasing the number of animals used in research studies. A toxicity model for combustion gases could also support researchers (civilian and military) in assessing potential fire gas problems associated with new products under development. However, few scientists have attempted to model toxicity of combined combustion gases, in part, due to the complexity of the fire scenario. Indeed, much is still being learned about the toxicity of individual gases.

By far, the leader in combustion gas modelling has been the National Institute of Standards and Technology (NIST, formerly the National Bureau of Standards), and their Building and Fire Research Laboratory (BFRL). Over a decade of research using rodents has produced a mathematical model (N-Gas) which can predict lethality, given that

concentrations of CO, CO_2, HCN, O_2, HCl and HBr are known (Levin, 1992). Last year, the Fire Science Branch of BFRL was disbanded, and the NIST effort of toxic gas modelling was assumed by the Department of Respiratory Research. Currently, the Department of Respiratory Research, through contractor JAYCOR, is incorporating a seventh gas, NO_2, into the model, using NO_2 lethality data collected at NIST. JAYCOR is also empirically validating the model at the Department of Respiratory Research (WRAIR Protocol M03-94).

As advanced as N-Gas is, the model still needs refinement. A major question about the model is whether it may be applicable to large animals, or even humans. Over fifty years of toxicological research of individual gases suggests that a size-dependent sensitivity to toxic gases appears to exist (Book, 1982; Carson *et al*, 1962). For example, while exposure to 100 ppm NO_2 for 15 minutes has been shown to cause significant elevations in extravascular lung water in rats (Stavert and Lehnert, 1990) and decreased dry/wet lung weight ratios in guinea pigs (WRAIR Protocol M02-88), it has been shown to cause no appreciable physiologic changes or demonstrable pathological lesions in sheep (Januszkiewicz *et al*, 1992). Moreover, while 500 ppm NO_2 for 20 minutes has been shown to cause significant perturbations in cardiopulmonary function and blood biochemistry, but no death, in sheep (Januszkiewicz and Mayorga, 1994), the same concentration-duration exposure would be expected to produce nearly 100% mortality in small-sized animals. This apparent sensitivity disparity between small and large animals may be based on respiration (more specifically, weight-specific minute ventilation) differences (Book, 1982; Phalen, 1984). Smaller sized animals, with high metabolic rate, breathe a greater volume of air, proportional to their body mass,

and, thus, may breathe in more toxin per unit time, compared to larger-sized animals, like sheep, whose respiratory physiology more approximates that of humans. Nevertheless, the question of extrapolation exists.

In addition to the scaling question, it is recognized that N-Gas is a lethality model which needs to be transitioned to an incapacitation model. While death is a final and all-or-none toxic endpoint, performance decrement is not. It is certainly important to know what the incapacitating thresholds are for toxic gases in a fire scenario. From a practical standpoint, while a fire within a crew compartment of a combat vehicle can be extinguished within milliseconds, toxic gases remain elevated for seconds to minutes, and gases may still be generated from smoldering materials long after the fire is extinguished. The Army is interested whether, given these circumstances, a crewmember can complete the mission, perform physical tasks, or advance to a safe position.

MILITARY RELEVANCE

In the early 1980's Congress mandated the Live Fire Testing Program in accordance with U.S. Code. Briefly, "Live Fire Law" requires that before manned weapon systems are fielded, or procured, they are to be evaluated with respect to vulnerability and survivability --- vulnerability with respect to the weapon systems capacity to withstand a foreign threat, and survivability with respect to incapacitation of crewmembers "behind defeated armor". The Department of Respiratory Research has been tasked to address survivability issues of this mission. While Combat Systems Test Activity performs the physical live fire tests and the Army Research Laboratory processes the data and calculates risk assessments, it is the

responsibility of the Department of Respiratory Research to develop guidance on risk (incapacitating effects) to the crew within a defeated vehicle, and validate risk assessments for Pentagon review. Guidance impacts on vulnerability/survivability evaluations, which helps in the decision process to determine whether a new system can be procured, or an old system needs to be re-engineered or decommissioned. The Department of Respiratory Research is the sole U.S. Army point of contact for toxic gas effects behind defeated armor.

The crew compartment of a defeated vehicle is a hostile environment. Spall (hot molten metal and fire), fragments, blast overpressure, and toxic gas are but a few general injury threat areas of concern. Airborne toxins and debris can be generated from burning of nitrogen-containing munitions and propellants (oxides of nitrogen), plastics and foams (formaldehyde and acrolein), teflon (perfluoroisobutylene), and others, as well as Halon pyrolysis (HCl, HF, HBr, phosgene). In addition, hydraulic fluid mist, radioactive material, glass, carbon and reactive particulates and other respirable chemicals and materials can foul the air. When crewmembers are not able to don personal protective gear, or available face masks do not appropriately impede the respirable toxins, the risk to the combat soldier is immediately apparent.

Guidance is based on many factors (literature, occupational standards, research, etc.). However, civilian occupational standards (OSHA) often do not apply, and research data are often sparse, because of the unique nature of military exposures (military: high concentration/short duration - acute toxicity; civilian: low concentration/long duration - chronic toxicity). Therefore, as required, gaps in the toxicological database are filled by departmental research. However, the task is large, considering the complexity of the

environment. While computer simulations and modelling are well under development in other areas of threat and injury (*ie*, blast overpressure), they have just been initiated in the toxic gas arena. Eventually a composite computer model, which addresses all of the systems hazards behind defeated armor, will be developed to predict injury and incapacitation, with diminished reliance on animal research.

HYPOTHESIS

The rodent LC_{50} (concentration which causes 50% death of an animal population) for combined gas exposure will cause negligible mortality in sheep. However, it may induce significant perturbations in vital function. If the hypothesis is correct, this research will constitute a first step in developing a scaling factor for the N-Gas model. Subsequently, a refined model can be tested empirically in a follow-up protocol.

OBJECTIVE

To evaluate pulmonary mechanics, hemodynamics, and blood biochemistry in sheep exposed to a combined gas mixture simulating, in part, gases generated during a simple combustion process.

JUSTIFICATION FOR PROPOSED ANIMAL MODEL

The sheep was selected as the experimental model in these studies for several reasons. Firstly, a large database on the cardiopulmonary function of both normal and airway-diseased sheep is available for comparative purposes. The ovine model has become popular

in respiratory research primarily because 1) sheep and humans have approximately the same lung size, 2) the lung is the primary "shock organ" for anaphylactic reactions in both species and 3) like humans, sheep tolerate pulmonary function maneuvers well in the conscious and unsedated state. Secondly, the ovine model has been used in many previous military-relevant toxicity studies. It is important to maintain consistency among the different studies in order to assess how one species can tolerate a variety of toxic insults. This is particularly important since humans can potentially encounter several different toxic insults simultaneously during various combat scenarios.

LITERATURE SEARCH

WRAIR Library-assisted literature searches (MEDLINE, TOXLINE, AGRICOLA, CHEMLIT, TOXICOL and PHARM conducted from December 1993 to February 1994, and DTIC #GOK02J, GOL20I, GOL35K) and subsequent to-date personal Current Contents searches revealed that the experiments as described in this proposal have not been conducted. Keywords/phrases include, but were not limited to, LC_{50} studies on the individual gases in rats and/or sheep and computer modelling of combustion gases and/or smoke effects in animals.

INVESTIGATOR EXPERIENCE

The Department of Respiratory Research has been involved in pulmonary research employing the ovine model for nearly a decade. The Department is fully equipped to perform the proposed studies safely while causing minimal discomfort to the animals. The

principal investigator of this proposal has extensive experience in cardiopulmonary research and surgical techniques, humane animal care, gas handling procedures, and has worked with the sheep as an experimental animal model since 1978. The principal investigator has authored/co-authored approximately 30 research publications using sheep, and has been involved as principal investigator/co-investigator in nearly as many approved protocols since coming to WRAIR in 1986. Co-investigator Smith, an on-site JAYCOR contractor, is an accomplished and published analytical chemist, and is currently the Principal Investigator on a WRAIR-approved rodent study to validate the N-Gas model. MAJ Nossov has been a veterinarian since 1982, and has had the responsibilities as Chief, Department of Animal Resources (Division of Veterinary Medicine). She is in the Laboratory Animal Residency Program at WRAIR, and has been a primary health care provider for the Department of Respiratory Research sheep population for over a year. Dr. Topper is a board-certified pathologist. While competent individuals from Division of Pathology and Division of Veterinary Medicine have been identified as Co-Investigators, the respective Divisions have historically provided a team approach, intellectually and practically, and have supported large animal experiments in full.

MATERIALS and METHODS

Animal Preparation. Twelve respiratory disease-free crossbred ewes (35-50 kg), essentially Q-Fever free, will be required for the study. Approximately eight animals can be identified in the Department of Animal Resources animal issue pool and can be transferred to this protocol. The balance will be obtained from the same commercial supplier as those in the

issue pool (Ovine Technologies, Inc., New Hope, PA). The sheep will be cared for in accordance with the principles in the Guide for the Care and Use of Laboratory Animals. They will be fed a small ruminant diet, have unlimited access to water and be housed in indoor runs in an environmentally stable atmosphere with controlled temperature and humidity.

During the 2 week quarantine period, the sheep will be re-tested for Q-Fever. In addition, the sheep will receive a complete physical examination by an attending veterinarian. This will include a complete blood chemistry and complete hematology evaluation to rule out occult disease. These studies will be repeated at the end of the study. The chemistry and hematology profiles will be performed through the Department of Clinical Pathology at WRAIR. The blood chemistry evaluation will include the following specific tests:

Sodium (Na)
Potassium (K)
Chloride (Cl)
Carbon dioxide (CO_2)
Glucose (Glu)
Blood urea nitrogen (BUN)
Creatinine (Cr)
Total protein
Albumin
Total bilirubin
Alkaline phosphatase (Alk P)
Lactate dehydrogenase (LDH)
Serum transferase (AST and ALT)

The hematology evaluation will include:

White blood cell count (WBC)
Red blood cell count (RBC)
Hemoglobin (Hb)
Hematocrit (HCT)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Plt)
Differential white blood cell count (Diff)

Animals will be evaluated by an attending Department of Respiratory Research veterinarian during the course of this study for any cyanosis, vomiting, diarrhea and dehydration. Heart rate, respiratory rate, temperature, and mucous membrane color and moisture will be recorded.

After the quarantine period, each sheep will be prepared with a chronic carotid artery loop at least one month prior to the experimentation. Prior to surgery the animals will have baseline hematocrit (hemoglobin) measured. Food will be withheld 24-48 hours before surgery. The animals will be allowed free access to water. All surgical procedures will be conducted using aseptic technique, primarily performed by competent Division of Veterinary Medicine professional and technical surgical support team. Briefly, the animal is prepared for surgery and placement of a catheter into the saphenous vein, by first administering 7-10 mg/kg ketamine into the jugular vein using a 20 gauge needle. Anesthesia is then induced with thiamyal sodium (10 mg/kg IV to effect). The trachea will be intubated with an endotracheal tube and a surgical plane of anesthesia (defined by heart rate, respiratory rate, noxious stimuli perception, *etc.*) and positive pressure ventilation will be maintained with 2-2.5% isoflurane and oxygen throughout the procedure. An orogastric tube will be placed to relieve rumen tympany. Intravenous support (electrolytes) will be initiated and continued until the animal has recovered. Buprenorphine (0.005 mg/kg, SQ), for analgesia, will be given immediately after induction of anesthesia, and every 12 hours post surgery, when necessary.

Sheep will be prepared with chronic carotid loops by the technique described by Bone *et al* (1962), with modifications as described by Lagutchik *et al* (1992). The surgical area will be closely clipped and prepared for aseptic surgery (povidone scrub and alcohol wash, *etc.*). Briefly, the surgical procedure first requires isolating the carotid artery from its surrounding tissue, including the common sheath also containing the vagus nerve and sympathetic trunk. The isolated carotid artery is then enveloped and sutured in a bipedicled skin tube and is allowed to heal for a one month period. Regarding post-operative care, a sterile non-adherent pad is placed between the loop and the skin of the neck to prevent adherence of the suture lines. An antiseptic povidone-iodine gel is applied to the loop, and the loop is aseptically bandaged for 3-4 days. Sutures are removed approximately 10 days after surgery. Antibiotics are not routinely required post surgery. Following the recovery period, the carotid artery exists as a "pull-like" externalized appendage which is easily accessed by simply piercing the few millimeters of skin surrounding the artery with a 19-22 gauge needle, or inserting an indwelling catheter. This chronic carotid artery loop technique greatly facilitates arterial blood sampling and pressure measurement and decreases the probability of infection and clotting of chronic arterial blood cannulas frequently encountered with repeated and prolonged blood sampling. At the end of the experimental day the needle/catheter is simply removed. Routinely, one suture is required to close the small wound site.

All sheep will be fasted 24 h prior to experimentation but allowed free access to water. On the day of an experiment, a sheep will be suspended in a sling attached to the inside of a large-animal metabolic cart. This procedure will minimally restrain the sheep and immobilize the head/neck region while imparting minimal discomfort (Coulson *et al*, 1989).

Ten-percent lidocaine spray (Xylocaine, Astra Pharmaceutical Products, Inc., West Roxbury, MA) will be used to topically anesthetize the nasal passages. A 7.5 mm ID foam-cuffed nasotracheal tube (Bivona, Inc., Gary, IN) will be lubricated with 2% viscous lidocaine (Barre-National, Inc., Baltimore, MD). A bronchoscope will then be used to transnasally pass the nasotracheal tube below the larynx for pulmonary mechanics and respiratory measurements.

The sheep will also be surgically prepared with venous and arterial catheters for blood sampling, and cardiovascular and core temperature measurements. Catheters will be aseptically placed using kits (Accuguide, Burron Medical, Inc., Bethlehem, PA) after local anesthesia with 2% lidocaine (Xylocaine, Astra Pharmaceutical Products, Inc., West Roxbury, MA). A percutaneous approach to the carotid artery in the loop will be made and a 14-gauge lateral hole catheter introduced and sutured into place. Similarly, an 8-French catheter with hemostasis valve will be introduced into the jugular vein, through which a 7-French thermodilution catheter is inserted and passed through the right heart into the pulmonary artery. Catheter patency will be maintained with sterile heparinized (100 U/ml) physiologic saline.

Pulmonary Mechanics and Respiration. A 4-French catheter pressure transducer (model PR-219, Millar Micro-Tip, Millar Instruments, Inc., Houston, TX) will be inserted through a nasotracheal tube side port and advanced to the distal end of the tube for trachea lateral pressure determinations. The non-intubated nasal passage will be topically anesthetized and a second catheter transducer (8-French, model PR-346, Millar Micro-Tip, Millar

Instruments, Inc., Houston, TX) will be passed transnasally to the esophagus for pleural pressure estimation. The pressure tracing from the esophageal transducer is accepted as pleural pressure when signals show a negative deflection upon inspiration and when clearly-defined cardiac oscillations are observed in the waveforms. Transpulmonary pressure is calculated as the difference between trachea lateral pressure and pleural pressure. A heated pneumotachometer (Series 3700, Hans-Rudolf Inc., Kansas City, KS) will be connected to the proximal end of the nasotracheal tube. Tidal volumes are derived from integrated flow signals. Isovolume technique is used to calculate lung resistance (R_L) from flow, volume and transpulmonary pressure signals while dynamic lung compliance (C_{dyn}) is derived from transpulmonary pressure and tidal volume signals at points of zero airflow. Inspired minute ventilation is calculated as the product of mean tidal volume and breathing rate. However, during exposure, inspired minute ventilation is calculated from inspired air volume, over time, measured with a turbine flowmeter (model VMM-2A, Sensormedics Corp., Anaheim, CA). The flowmeter is positioned in-line between the test gas and the animal.

Hemodynamics. Fluid-filled strain gauges (model P23XL, Spectramed Inc., Oxnard, CA) will be connected to the thermodilution and arterial catheters to monitor heart rate, and carotid artery (Pa), right atrium (Pra), pulmonary artery (Ppa) and pulmonary artery wedge (Ppw) pressures. Catheter positioning is verified by characteristic blood pressure waveforms. The thermodilution catheter is positioned in the pulmonary artery such that a wedge pressure (pulmonary artery occlusion pressure) is obtained upon balloon inflation. A computer (model SP1445, Spectramed Inc., Oxnard, CA) will be used to measure cardiac output (Q_T)

by thermodilution technique. A minimum of 3 measurements will be made per sampling time and the values averaged. The computer also displays a core temperature measurement. Systemic vascular resistance is calculated as (Pa - Pra)/Q_T and pulmonary vascular resistance as (Ppa - Ppw)/Q_T. Hemodynamic and respiratory variables are collected using a direct writing recorder (model 2800S, Gould, Inc., Cleveland, OH).

Other Measurements. Arterial and mixed-venous blood samples will be anaerobically-collected for blood gas and chemistry analyses. One-ml each of arterial and venous blood will be analyzed with an automated pH/blood gas analyzer (model 1306, Instrumentation Laboratories, Lexington, MA), oximeter (model OSM3, Radiometer, Copenhagen, Denmark), glucose/L-lactate (Yellow Springs Instrument Co., Yellow Springs, OH) and sodium/potassium (STARLYTE II, Pharmacia Diagnostics, Inc., Fairfield, NJ) analyzers. The analyzers will measure whole blood pH, oxygen and carbon dioxide partial pressures, plasma bicarbonate, total hemoglobin, methemoglobin, sodium, potassium, glucose, lactate and other important blood constituents. An additional 5 ml venous blood will be collected each sampling time and examined for signs of oxidative stress (free radical generation, lipid peroxidation, red-ox ratios, etc). After the final sampling time, an additional 20 ml of blood will be collected, and chemistry and hematology profiles will be performed by the Clinical Pathology Laboratory, for comparison to pre-exposure values. Total blood volume withdrawn over the pre exposure and 24-hour observation period will likely not exceed 55 ml. Hematocrit will be determined by centrifugation. Oxygen consumption will be calculated as the product of the arterio-venous oxygen content difference and Q_T.

Pathology. Twenty-four hours after the exposure, the animals will be euthanized humanely by anesthesia with a diazepam-ketamine overdose (approximately 30 mg/kg ketamine and 1.5 mg/kg diazepam) followed by exsanguination. The diazepam-ketamine overdose represents a 50/50 mixture, by volume, of commercially prepared drugs, and approximately a 12 ml total volume for a 50 kg sheep. This mixture was selected over a barbiturate overdose, or use of a commercially prepared euthanasia solution, since the latter agents may artificially induce lung edema, a toxicologic endpoint of these experiments. Subsequent to euthanasia, lungs will be subjected to histopathological analyses. Examination and preparation of lung tissue will be conducted by a veterinary pathologist at WRAIR who will have no prior knowledge of the treatment groups. Initially, the in situ lungs will be examined and reportable lesions and changes will be properly identified. Subsequently, gravimetric analyses of the lung tissue will be performed in order to assess pulmonary edema development. Gravimetric determinations will be made as follows. Using a method to keep the lungs inflated to their thoracic cavity dimensions, the entire intact lungs will be removed from the thoracic cavity and lung wet weight/body weight ratios obtained. Lung sections from pre-determined lung lobes also will be taken and gently blotted dry and weighed. Then, the samples will be dried to a constant dry weight. The lung (dry/wet) weight ratio will be calculated from the above values.

Immediately subsequent to obtaining lung samples for gravimetric analyses, the lungs will be prepared for histologic examination. Lung fixation will be accomplished with 4% formaldehyde : 1% glutaraldehyde phosphate buffer solution by perfusion through the trachea. The perfusion pressures will not exceed 10 cmH_20. Tissue blocks will then be cut

from pre-determined lung sample sites. Routine light microscopy tissue preparation and evaluations will be used with special histology procedures applied as required. The lung tissue will then be examined for typical pathological features associated with oxidant/combustion gas exposure. These features include, but are not limited to, capillary congestion, interstitial edema, intraalveolar edema, atelectasis, alveolar hemorrhage, hyaline membrane formation, cell hyperplasia, fibrosis and inflammatory cell infiltration (eosinophils, neutrophils, macrophages). The relative intensity of these pathological features will be graded on a 0-4 scale with "0"indicating that the condition was not observed and "4" indicating extensive damage. Additionally, the distribution of the pathologic conditions will be graded on an arbitrary 1-3 scale with "1" indicating a focal lesion, "2" indicating a multifocal lesion and "3"indicating a diffuse lesion.

Exposures. For these experiments, the test gases will not be generated by combustion, rather reagent grade pure gases will be individually metered into a mixing chamber and delivered to the sheep. During the exposures, both the animal and the gas delivery system will be contained in a walk-in sized fume hood. The hood will be maintained under negative pressure (to perimeter hallways and laboratories) and will be capable of approximately two air-changes per minute. In addition, the exhaust gas will be passed through an industrial gas scrubber (Duall Model ET-50) before exiting the building. This procedure should assure >90% reduction in concentration reactive gases in the exhaust. Ambient air quality in the laboratory will be monitored during experiments, via gas analyzers and other personal monitoring equipment, to ensure that ambient levels of gases do not exceed OSHA limits.

Only the Principal Investigator, donned with a positive-pressure, self-contained breathing apparatus (SCBA), will be in the immediate area of exposure. Other investigators, outside the exposure area, will have ready access to two wall-mounted SCBA.

Sheep will be exposed to either medical-grade air (control) or the combined gas mixture. The combined gas mixture, approaching an LC_{50} value for rats, will be comprised of CO, CO_2, NO and NO_2. Exposure durations will be 30 min. Gas concentrations will be determined following JAYCOR validation experiments. The sheep will breathe the test gas *ad libitum* through a two-way non-rebreathing valve from a dynamic exposure system. A plastic canine anesthesia mask with a rubber diaphragm (A.J. Buck & Sons, Inc., Cockeysville, MD), modified with a 3-cm ID inlet, will be used for these nose-only exposures. The gas delivery system is composed of glass, Teflon or Teflon-conditioned components. The test gas will reach 99% of the nominal concentration at the mask inlet within 15 sec after an in-line solenoid valve is opened. Gas concentrations will be continuously monitored throughout the exposure. Nitrogen dioxide and NO concentrations will be measured with a dual beam IR-UV spectrophotometer (Binos). Carbon monoxide and CO_2 concentrations will be measured by non-dispersive infrared spectroscopy (Binos and Ametek, respectively). The toxic gas doses presented to the animal will be estimated as the product of gas concentration (mg/l), inspired minute ventilation (l/min) and exposure duration (min), and normalized to body weight (kg).

Data Analysis. Data will be entered onto computer spreadsheet format and subjected to statistical comparisons. All comparisons will be conducted using raw data. A one-way and

two-way analysis of variance (ANOVA) will be performed for effects of gas concentration and effects over time, respectively. If significance is found, the ANOVA will be followed by a Duncan's multiple-range test to determine where the significant differences exist. Significance will be accepted at the 95% ($p < 0.05$) confidence level.

EXPERIMENTAL

All surgical procedures will be conducted using aseptic technique while imparting minimal discomfort to the animals. The surgical facilities will be provided by the Division of Veterinary Medicine.

Twelve sheep will be required for the completion of the proposed study. Six sheep will be used in each of the two treatment groups (control and combined gas). In the exposure protocol, pulmonary function, hemodynamics and blood chemistry analyses will be performed immediately before exposure (PRE), immediately after exposure (POST), and at 1, 4 and 24 hours after exposure. Inspired minute ventilation will also be measured during exposure. The nasotracheal tube will be removed between the 4- and 24-h sampling times and re-introduced for 24-h post-exposure sampling. In addition, the nasotracheal tube will be removed 1 h prior to exposure and re-inserted immediately post exposure for these experiments. After the final sampling time, the animals will be euthanized for the purpose of histopathological analyses.

The sheep will be examined in groups of three, with each animal in the group given one of the two randomly-selected exposure treatments. After six of the animals have been examined, three sheep in each of the two treatment groups, the data will be subjected to

statistical analyses. If the individual variances are smaller than expected and statistically significant differences are found among the different sampling times in a particular treatment group, no further exposures in that treatment group will be required. If the mean values among the sampling times in the treatment group are found to be marginally significant, then the final three animals will be examined in that treatment group. Finally, if the individual variances are much larger than expected, the experiments will be terminated and the treatment methods will be re-evaluated. The above measures will be taken in order to eliminate unnecessary use of the laboratory animals.

Bronchoconstriction, or narrowing of the airways, may be the only stress potentially experienced by sheep in this protocol. It is an important physiological endpoint of this study and, unless lung resistance is unusually high and coupled with other physiologic/behavioral signs of pain/distress, bronchoconstriction will not be relieved. Bronchoconstriction, while not painful, can be stressful. However, it is used as a controlled and titrated variable in standard bronchoprovocation tests performed on humans, without analgesia, in pulmonary clinics throughout the country. Experience in our laboratory has revealed that sheep possess the capacity to withstand mild bronchoconstriction, as no changes in adaptive respiratory pattern (wheezing, dyspnoea) are observed. Otherwise, if it is apparent that undue stress or pain (dyspnoea, lameness, recumbency, decreased appetite, *etc.)* is presented to the sheep at any time (evaluated by the attending Department of Respiratory Research veterinarian and/or responsible investigators) the experiments will be terminated, appropriate relief measures (supportive ventilation with oxygen, pharmacologic intervention - *ie* methylene blue, bronchodilators, analgesics, steroids, *etc.*, food supplementation) will be taken in

accordance with Division of Veterinary Medicine guidance, and the LACUC committee will be informed.

REFERENCES

Bone JF, Metcalf J and Parer JT, Surgical preparation of a carotid loop in sheep. Am. J. Vet. Res., 23 (1962) 1113.

Book SA, Scaling toxicity from laboratory animals to people: An example with nitrogen dioxide. J. Toxicol. Environ. Health, 9 (1982) 719.

Carson TR, Rosenholtz MS, Wilinski FT and Weeks MH, The response of animals inhaling nitrogen dioxide for single, short-term exposures. Am. Ind. Hyg. Assoc. J., 23 (1962) 457.

Coulson NM, Januszkiewicz AJ, Dodd KT and Ripple GR, The cardiorespiratory effects of diazepam-ketamine and xylazine-ketamine anesthetic combinations in sheep. Lab. Anim. Sci., 39 (1989) 591.

Januszkiewicz AJ and Mayorga MA, Nitrogen dioxide-induced acute lung injury in sheep. Toxicol., 89 (1994) 279.

Januszkiewicz AJ, Snapper JR, Sturgis JW, Rayburn DB, Dodd KT, Phillips YY, Ripple GR, Sharpnack DD, Coulson NM and Bley JA, Pathophysiologic responses of sheep to brief high-level nitrogen dioxide exposure. Inhal. Toxicol., 4 (1992) 359.

Lagutchik MS, Bley JA, Sturgis JW and Martin DG, Review of the carotid artery loop procedure in sheep. J. Invest Surg., (1992) 79.

Levin BC, The development of a new small scale smoke toxicity test method and its comparison with real scale fire tests. Toxicol. Lett., 64/65 (1992) 257.

Loick HM, Traber LD, Tokyay R, Linares HA, Prien T and Traber DL, Mechanical alteration of blood flow in smoked and unsmoked lung areas after inhalation injury. J. Appl. Physiol., 72 (1992) 1692.

Phalen RF, Inhalation Studies : Foundations and Techniques. CRC Press, Boca Raton, Fla., 1984.

Stavert DM and Lehnert BE, Nitric oxide and nitrogen dioxide as inducers of acute pulmonary injury when inhaled at relatively high concentrations for brief periods. Inhal. Toxicol., 2 (1990) 53.

Wang CZ, Li A and Yang ZC, The pathophysiology of carbon monoxide poisoning and acute respiratory failure in a sheep model with smoke inhalation injury. Chest, 97 (1990) 736.

Data Files by Exposure Group

GROUP: AIR CONTROLS			Tag 23-30, controls 25 & 29				
DATE: 25 August 1994						ANIMAL WEIGHTS	
						tag #	grams
Time, min	O2%	ave O2%	CO2,%	Ave CO2%		23	411
0	20.88		0.17			24	360
1	20.83	20.86	0.2	0.19		25	332
2	20.78	20.81	0.23	0.22		26	369
3	20.75	20.77	0.26	0.25		27	337
4	20.71	20.73	0.28	0.27		28	366
5	20.68	20.70	0.31	0.30		29	346
6	20.66	20.67	0.33	0.32		30	377
7	20.62	20.64	0.36	0.35			
8	20.6	20.61	0.38	0.37			
9	20.57	20.59	0.4	0.39			
10	20.52	20.55	0.44	0.42			
11	20.49	20.51	0.46	0.45			
12	20.47	20.48	0.48	0.47			
13	20.45	20.46	0.51	0.50			
14	20.43	20.44	0.53	0.52			
15	20.4	20.42	0.55	0.54			
16	20.38	20.39	0.56	0.56			
17	20.37	20.38	0.57	0.57			
18	20.35	20.36	0.6	0.59			
19	20.32	20.34	0.62	0.61			
20	20.3	20.31	0.63	0.63			
21	20.28	20.29	0.65	0.64			
22	20.27	20.28	0.66	0.66			
23	20.26	20.27	0.67	0.67			
24	20.24	20.25	0.67	0.67			
25	20.23	20.24	0.68	0.68			
26	20.23	20.23	0.69	0.69			
27	20.22	20.23	0.7	0.70			
28	20.21	20.22	0.71	0.71			
29	20.2	20.21	0.71	0.71			
30	20.19	20.20	0.71	0.71			

20.4 0.51

	Syringe#	Time	Animal#	tHb	SAT	HbCO	MetHb
	1	0	28				
	2	0	25				
	3	5	28				
	4	10	25				
	6	15	25				
	5	30	28				
	7	60	28				
	8	120	25				

O2ct	HbO2	RHb	O2cap	pH	pCO2	pO2	HCO-	TCO2	BEb
				7.44	42.2	91	28.9	30.2	4.9
				7.45	39.5	90	28	29.2	4.4
				7.44	41.3	90	28.6	29.9	4.7
				7.46	37.3	91	26.8	28	3.6
				7.46	37.9	87	27.1	28.2	3.8
				7.45	41.5	81	29.1	30.4	5.2
				7.45	40.4	90	28.6	29.9	4.9
				7.44	41	92	28.2	29.5	4.3
			ave=>>>	*7.45*	*40.14*	*89.00*	*28.16*	*29.41*	*4.48*

SBC	BEecf	%sO2c	wt.
28.7	4.6	97.3	343
28.3	3.8	97.3	332
28.6	4.3	97.2	
27.8	2.8	97.5	
27.9	3	97.2	
28.9	4.9	96.3	
28.8	4.5	97.3	
28.3	3.9	97.4	
28.41	*3.98*	*97.19*	

CHAMBER CONDITIONS VS. TIME				EXPOSURE GROUP id:				
TAG NUMBERS:								
TIME, min	TEMP, F	RH, %	O2 %	CO2 %	CO, PPM	CO, %	NO2, PPM	
0		74	20.03	5.03				
1			20.02	4.62				
2			19.98	4.72				
3			19.93	4.85				
4			19.88	5.01				
5			19.83	5.16				
6			19.75	5.3				
7			19.69	5.34				
8			19.635	5.385				
9			19.58	5.43				
10			19.56	5.34				
11			19.56	5.27				
12			19.56	5.195				
13			19.57	5.12				
14			19.52	5.17				
15			19.48	5.19				
16			19.47	5.2				
17			19.45	5.21				
18			19.41	5.21				
19			19.36	5.25				
20			19.31	5.3				
21			19.27	5.31				
22			19.24	5.31				
23			19.19	5.35				
24			19.16	5.38				
25			19.14	5.36				
26			19.11	5.37				
27			19.08	5.38				
28			19.06	5.38				
29			19.04	5.39				
30			19.01	5.39				
35			20.27	0.07				
45			20.8	0.06				

		BLOOD CHEMISTRY VS TIME & BY ANIMAL						
TIME,min	Animal#	pH	pCO2	pO2	HCO-	TCO2	BEb	SBC
3	7	7.339	54.5	106	29.7	31.4	3.2	27.4
10	12	7.331	51	106	27.2	28.8	1	25.7
15	7	7.34	53.6	102	29.2	30.9	2.8	27.1
30	12	7.341	51.7	91	28.3	29.8	2.1	26.5
60	7	7.411	45.3	90	29.1	30.5	4.3	28.3
120	12	7.427	43.1	92	28.7	30	4.4	28.3
0	7	7.456	39.6	80	28.2	29.4	4.6	28.5
0	12	7.471	41.3	81	30.5	31.7	6.8	30.2
5	7	7.409	48.8	110	31.2	32.7	5.9	29.6

BEefc	%sO2c	tHb	SAT	HbCO	MetHb	O2ct	HbO2	RHb%
3.7	97.6							
1.1	97.6							
3.3	97.3							
2.3	96.3							
4.3	96.9							
4.2	97.3							
4.1	96.4							
6.6	96.6							
6.3	98.2							

temp, cor	O2cap	Animal wt.	

1-Sep		2000ppmCO, in air					
	Time,min	O2%	CO2%	CO,ppm	k, CO	k,%CO	"FED"
	0	21.21	0.52	2010	2010	1.46%	0.30
	1	20.11	0.62	2020	4030	2.92%	0.37
	2	20.07	0.66	2010	6040	4.38%	0.37
	3	20.03	0.7	1990	8030	5.82%	0.37
	4	19.96	0.76	2020	10050	7.28%	0.38
	5	19.89	0.82	2010	12060	8.74%	0.39
	6	19.81	0.89	2010	14070	10.20%	0.40
	7	19.77	0.93	2010	16080	11.65%	0.40
	8	19.7	1	1990	18070	13.09%	0.40
	9	19.66	1.04	2040	20110	14.57%	0.41
	10	19.6	1.08	2030	22140	16.04%	0.42
	11	19.56	1.14	2040	24180	17.52%	0.42
	12	19.51	1.21	2030	26210	18.99%	0.43
	13	19.45	1.27	2015	28225	20.45%	0.43
	14	19.42	1.31	1990	30215	21.89%	0.43
	15	19.39	1.34	1970	32185	23.32%	0.43
	16	19.31	1.44	2000	34185	24.77%	0.44
	17	19.31	1.41	2010	36195	26.23%	0.44
	18	19.3	1.46	2040	38235	27.71%	0.45
	19	19.27	1.49	2060	40295	29.20%	0.46
	20	19.24	1.54	2040	42335	30.68%	0.46
	21	19.19	1.58	2010	44345	32.13%	0.46
	22	19.17	1.6	1990	46335	33.58%	0.46
	23	19.14	1.63	1970	48305	35.00%	0.45
	24	19.12	1.68	1980	50285	36.44%	0.46
	25	19.1	1.7	1960	52245	37.86%	0.46
	26	19.08	1.72	1940	54185	39.26%	0.46
	27	19.05	1.75	1990	56175	40.71%	0.47
	28	19.02	1.78	2030	58205	42.18%	0.48
	29	18.98	1.84	2050	60255	43.66%	0.49
	30	18.96	1.88	2030	62285	45.13%	0.48
	AVE>>>	19.50	1.28	2009.19			

	A-7				Animal Number		
	Date	31	32	34	35	36	37
	8/24/94	327	304	288	307	304	312
	8/26/94	347	313	299	323	315	330
	8/28/94	367	331	317	332	331	347
	8/29/94	380	341	326	343	343	363
	8/30/94	392	348	334	357	349	368
	8/31/94	392	349	329	353	352	368
	9/1/94	406	361	344	372	363	371
	9/2/94	403	354	338	363	363	EUTH
	9/4/94	412	366	347	370	372	
	9/5/94	420	372	351	373	380	
	9/6/94	431	378	361	381	383	
	9/7/94	431	382	362	382	393	
	9/8/94	436	385	364	383	394	
	9/9/94	439	389	372	386	405	
	9/10/94	443	392	368	376	397	
	9/11/94	447	390	376	358	406	
	9/12/94	461	399	381	355	418	
	9/13/94	464	402	392	343	425	
	9/14/94	470	408	363	345	431	
	9/15/94	473	409	388	337	434	

Syringe#	Time	Animal#	tHb	SAT	HbCO	MetHb
1	0	36	14	86.1	0	0
			14.4	83.7	0	0
			13.8	85.4	0	0
		AVE >>>	14.07	85.07	0.00	0.00
2	0	38	14.1	86.6	0	0
			13.9	85.4	0	0
		AVE >>>	14	86	0	0
3	5	36	14	94.1	23.8	0
			14	94.8	25.2	0.1
		AVE >>>	14	94.45	24.5	0.05
4	10	38	13.5	94.1	48.1	0
	(smp took 4 min)		13.6	93.4	48.7	0
		AVE >>>	13.55	93.75	48.4	0
5	15	36	13	92.8	58.1	0
	(smp took 4 min)		13.4	94.4	59.5	0
		AVE >>>	13.2	93.6	58.8	0
6	30	38	14.7	85.3	66.4	0.2
	(smp took 5 min)		14.8	84.7	66.8	0.2
		AVE >>>	14.75	85	66.6	0.2
7	60	36	13.3	93.5	26.8	0
	(cardiac punc)		12.2	94.5	27.6	0.3
		AVE >>>	12.75	94	27.2	0.15
8	120	38	14.9	94	11.1	0.5
	(prob. cardiac punc		15	939	10.9	0.6
		AVE >>>	14.95	516.5	11	0.55

SUMMARY OF SAMPLE SIZE TO HEMOGLOBIN READINGS, ALL SAMPLES AR

Size,ml	tHb	SAT	HbCO	MetHb	O2ct
0.25	14.5	17.3	17.3	0.3	2.9

			14	9	17.4	0.2	1.5
		AVE>>	14.25	13.15	17.35	0.25	2.2
		0.5	12.7	3.1	17.2	0.2	0.5
			13.3	1	17.2	0.3	0.1
		AVE >>>	13	2.05	17.2	0.25	0.3
		0.75	12.9	0.9	17.2	0.3	0.1
			13.3	0.9	17.2	0.3	0.4
		AVE >>>	13.1	0.9	17.2	0.3	0.25
		1.00	13.2	10.2	18.2	0.2	1.5
			12.6	10.7	18.1	0.2	1.5
			12.9	10.45	18.15	0.2	1.5
		NOTE: SAMPLES WERE DRAWN AT SAME TIME (?) AND RUN IN S					
38	39	AVG	SD				
295	314	306.375	17.21243				
304	339	321.25	19.09188				
321	355	337.625	20.20564				
328	366	348.75	20.04949				
339	380	358.375	21.47049				
341	375	357.375	23.44071				
353	399	371.125	22.26806				
341	EUTH	360.3333	23.77744				
357		370.6667	24.09772				
361		376.1667	23.4215				
373		384.5	24.41857				
377		387.8333	24.24046				
380		390.3333	26.83033				
379		395	30.50519				
380		392.6667	35.93698				
386		393.8333	39.93328				
394		401.3333	45.43127				
396		403.6667	45.60446				
395		402	#DIV/0!				
400		406.8333	#DIV/0!				

O2ct	HbO2	RHb	O2cap	pH	pCO2	pO2	HCO-
16.8	86.1	13.9	19.5	7.395	42.2	80	26.1
16.8	83.7	16.3	20				
16.4	85.4	14.6	19.2				
16.67	85.07	14.93	19.57				
17	86.6	13.4	19.6	7.315	39	104	20.1
16.5	85.4	14.6	19.3	7.314	39.5	101	20.3
16.75	86	14	19.45	7.3145	39.25	102.5	20.2
14	71.7	4.5	14.8	7.43	41.3	85	27.7
13.8	70.8	3.9	14.5	7.428	42.9	77	28.6
13.9	71.25	4.2	14.65	7.429	42.1	81	28.15
9.2	48.8	3.1	9.7	7.401	39.2	58	24.6
9.1	47.9	3.4	9.7	7.393	40.1	60	24.7
9.15	48.35	3.25	9.7	7.397	39.65	59	24.65
7	38.9	3	7.6	7.402	31.8	53	20
7.1	38.2	2.3	7.5	7.393	32.6	58	20
7.05	38.55	2.65	7.55	7.3975	32.2	55.5	20
5.8	28.5	4.9	6.8	6.802	38.6	57	6.1
5.8	28	5	6.8	6.797	38.6	64	6
5.8	28.25	4.95	6.8	6.7995	38.6	60.5	6.05
12.7	68.5	4.7	13.5	7.292	37.7	80	18.4
11.5	68.1	4	12.2	7.302	37.9	80	18.9
12.1	68.3	4.35	12.85	7.297	37.8	80	18.65
17.2	83.1	5.3	18.3	7.362	39.3	100	22.5
17.3	83.1	5.4	18.5	7.361	39.7	97	22.7
17.25	83.1	5.35	18.4	7.3615	39.5	98.5	22.6
E FROM CARDIAC PUNCTURE OF ANIMAL # 36							
HbO2	Rhb	O2cap					
14.2	68.2	16.6					

7.5	74.9	16					
10.85	71.55	16.3					
2.6	80	14.6					
0.8	81.7	15.3					
1.7	80.85	14.95					
?	?	?					
0.8	81.7	15.3					
0.8	81.7	15.3					
8.4	73.2	15					
8.8	72.9	14.3					
8.6	73.05	14.65					
EQUENCE, SMALLEST TO LARGEST							

TCO2	BEb	SBC	BEecf	%sO2c
27.4	1.6	26.1	1.1	95.5
21.3	-5	21	-6.2	97.4
21.5	-4.9	21.1	-6.1	97.2
21.4	-4.95	21.05	-6.15	97.3
28.9	3.5	27.7	3.1	96.7
29.9	4.3	28.2	4.1	95.5
29.4	3.9	27.95	3.6	96.1
25.8	0.5	25.1	-0.4	89.8
25.9	0.4	25	-0.4	90.2
25.85	0.45	25.05	-0.4	90
21	-3.1	22.2	-4.9	87.4
21	-6	22.1	-5.1	90
21	-4.55	22.15	-5	88.7
7.3	-27.3	2.1	-28.5	59.5
7.2	-28	2.2	-28.7	67.2
7.25	-27.65	2.15	-28.6	63.35
19.5	-6.9	19.4	-8.4	94.3
20.1	-6.3	19.9	-7.7	94.5
19.8	-6.6	19.65	-8.05	94.4
23.7	-2	23.4	-3.1	97.5
23.9	-1.9	23.4	-2.9	97.3
23.8	-1.95	23.4	-3	97.4

EXPOSURE GROUP: High CO in air								
DATE: 7 Sept 1994				TAG#: 20, 39-46, controls 43 & 20				
Time, min	time (c)	O2%	O2%, real	CO2%	CO2%,(r)	CO,ppm	CO,(c)	"k", CO
0		20.3		0.28		4580		
1	1	20.31	20.31	0.29	0.29	4500	4540	4540
2	2	20.2	20.26	0.4	0.35	4500	4500	9040
3	3	20.1	20.15	0.51	0.46	4510	4505	13545
4	4	20.06	20.08	0.54	0.53	4570	4540	18085
5	5	20.03	20.05	0.59	0.57	4550	4560	22645
6	6	19.99	20.01	0.64	0.62	4520	4535	27180
7	7	19.96	19.98	0.69	0.67	4505	4512.5	31692.5
8	8	19.94	19.95	0.75	0.72	4490	4497.5	36190
9	9	19.94	19.94	0.76	0.76	4460	4475	40665
10	10	19.93	19.94	0.78	0.77	4460	4460	45125
11	11	19.91	19.92	0.81	0.80	4450	4455	49580
12	12	19.9	19.91	0.83	0.82	4450	4450	54030
13	13	19.88	19.89	0.85	0.84	4440	4445	58475
14	14	19.86	19.87	0.88	0.87	4430	4435	62910
15	15	19.85	19.86	0.89	0.89	4420	4425	67335
16	16	19.84	19.85	0.92	0.91	4405	4412.5	71747.5
17	17	19.83	19.84	0.95	0.94	4390	4397.5	76145
18	18	19.83	19.83	0.95	0.95	4420	4405	80550
19	19	19.82	19.83	0.96	0.96	4490	4455	85005
20	20	19.81	19.82	0.97	0.97	4540	4515	89520
21	21	19.8	19.81	0.97	0.97	4580	4560	94080
22	22	19.8	19.80	0.99	0.98	4590	4585	98665
23	23	19.8	19.80	1.01	1.00	4600	4595	103260
24	24	19.78	19.79	1.03	1.02	4590	4595	107855
25	25	19.75	19.77	1.05	1.04	4580	4585	112440
26	26	19.74	19.75	1.06	1.06	4580	4580	117020
27	27	19.74	19.74	1.07	1.07	4580	4580	121600
28	28	19.73	19.74	1.07	1.07	4580	4580	126180
29	29	19.73	19.73	1.08	1.08	4580	4580	130760
30	30	19.72	19.73	1.09	1.09	4580	4580	135340
			19.90		0.83	ave>>>>	4511	

				Syringe#	Time	Animal#	tHb	SAT
				1	0	43	15.4	83.2
% LC50	CUM LC50						15.7	83.2
						AVE=>>>	15.55	83.2
3%								
7%				2	0	20	13.7	81.4
10%							14.1	82.3
13%						AVE=>>>	13.9	81.85
16%								
20%								
23%				5	20	43	17	0
26%				#43 removed at 20 min			18.1	0
29%				sampled by IC			17	0
33%						AVE=>>>	17.37	0.00
36%								
39%					30	20	18.9	2.8
42%				#20 removed at 30 min			18.7	5.3
46%				sampled by IC		AVE=>>>	18.8	4.05
49%								
52%						45	15.7	5.3
55%				died during exposure, IC			15.9	0
58%						AVE=>>>	15.8	2.65
62%								
65%						40	17	0
68%				died during exp, IC			17	0
71%						AVE=>>>	17	0
75%								
78%						39	17.3	6.4
81%				died during exp., IC			17.3	0
85%						AVE=>>>	17.3	3.2
88%								
91%						46	16.4	0
95%				died during exp., IC			18.5	0
98%						AVE=>>>	17.45	0

HbCO	MetHb	O2ct	HbO2	RHb	O2cap	pH	pCO2	pO2
0	0	17.8	83.2	16.8	21.4	7.352	37.5	73
0	0	18.2	83.2	16.8	21.8	7.373	45.7	82
0	0	18	83.2	16.8	21.6	7.3625	41.6	77.5
0	0	15.5	81.4	18.6	19	7.414	40.3	95
0	0	16.1	82.3	17.8	19.8	7.411	40.3	99
0	0	15.8	81.85	18.2	19.4	7.4125	40.3	97
79.3	0.3	0	0	20.4	4.8	6.735	90.9	1
79.7	0.2	0	0	20.1	5.1	6.734	88.5	2
79.4	0.2	0	0	20.4	4.8			
79.47	0.23	0.00	0.00	20.30	4.90	6.7345	89.7	1.5
72.4	0.2	0.2	0.8	26.6	7.2	6.796	43.3	3
72.3	0.2	0.4	1.5	26	7.1	6.791	44.3	7
72.35	0.2	0.3	1.15	26.3	7.15	6.7935	43.8	5
81.5	0.3	0.2	1	17.2	4			
81.6	0.3	0	0	18.1	4			
81.55	0.3	0.1	0.5	17.65	4	6.638	74.6	137
82.2	0.3	0	0	17.5	4.1	6.488	73.9	4
82.3	0.3	0	0	17.4	4.1	6.48	75.3	4
82.25	0.3	0	0	17.45	4.1	6.484	74.6	4
81.1	0.3	0.3	1.2	17.4	4.5			
81.2	0.3	0	0	18.5	4.4			
81.15	0.3	0.15	0.6	17.95	4.45	6.583	70.3	7
79.5	0.3	0	0	20.2	4.6	6.511	113	4
79.7	0.3	0	0	20	5.1	6.504	108.2	5
79.6	0.3	0	0	20.1	4.85	6.5075	110.6	4.5

HCO-	TCO2	BEb	SBC	BEecf	%sO2c			
21	22.2	-3.4	22.1	-4.7	93.7			
26.9	28.3	1.7	26.2	1.5	95.6	wt.		
23.95	25.25	-0.85	24.15	-1.6	94.65			
26	27.2	1.9	26.4	1.2	97.5			
25.8	27.1	1.7	26.3	1	97.7			
25.9	27.15	1.8	26.35	1.1	97.6			
12.3	15.1	-24.4	3.1	-23.5	0.4			Animal we
12	14.7	-24.7	2.9	-23.8	0.7			tag#
								20
12.15	14.9	-24.55	3	-23.65	0.55			39
								40
6.7	8.1	-27.4	0.7	-28	1			41
6.8	8.2	-27.5	0.7	-28	2.4			42
6.75	8.15	-27.45	0.7	-28	1.7			43
								44
								45
								46
8.1	10.3	-30	1.4	-29.3	91.4			
5.7	7.9	-35.3	-5.4	-34.1	0.9			
5.7	8	-35.5	-5.5	-34.2	1			
5.7	7.95	-35.4	-5.45	-34.15	0.95			
6.7	8.9	-32.2	-3	-31.5	2			
9.1	12.6	-32	-2.8	-30.3	1			
8.6	11.9	-32.6	-3.3	-30.9	1.2			
8.85	12.25	-32.3	-3.05	-30.6	1.1			

eights		
wt		
340		
404		
435		
433	died before exp	
367		
415		
390		
404		
396		

EXPOSURE GROUP: 5% C02 & 2500 PPM CO									
DATE: 9 SEPTEMBER				TAG#: 47 THRU 52, CONTROLS 50&52					
TIME	time (c)	O2%	O2% (c)	CO2%	CO2%, (c)	CO,ppm	CO (c)	CO, cumk	CO,%LC5(
0		19.6		5.39		2550			
1	1	19.44	19.52	5.14	5.27	2270	2410	2410	2%
2	2	19.35	19.40	5.2	5.17	2230	2250	4660	3%
3	3	19.33	19.34	5.28	5.24	2190	2210	6870	5%
4	4	19.29	19.31	5.32	5.30	2150	2170	9040	7%
5	5	19.23	19.26	5.32	5.32	2150	2150	11190	8%
6	6	19.16	19.20	5.32	5.32	2150	2150	13340	10%
7	7	19.12	19.14	5.34	5.33	2150	2150	15490	11%
8	8	19.09	19.11	5.36	5.35	2140	2145	17635	13%
9	9	19.05	19.07	5.39	5.38	2320	2230	19865	14%
10	10	19	19.03	5.41	5.40	2510	2415	22280	16%
11	11	18.95	18.98	5.46	5.44	2450	2480	24760	18%
12	12	18.9	18.93	5.5	5.48	2370	2410	27170	20%
13	13	18.85	18.88	5.56	5.53	2360	2365	29535	21%
14	14	18.78	18.82	5.61	5.59	2350	2355	31890	23%
15	15	18.76	18.77	5.63	5.62	2340	2345	34235	25%
16	16	18.73	18.75	5.67	5.65	2340	2340	36575	27%
17	17	18.71	18.72	5.69	5.68	2330	2335	38910	28%
18	18	18.68	18.70	5.71	5.70	2340	2335	41245	30%
19	19	18.65	18.67	5.73	5.72	2350	2345	43590	32%
20	20	18.62	18.64	5.75	5.74	2360	2355	45945	33%
21	21	18.6	18.61	5.78	5.77	2370	2365	48310	35%
22	22	18.57	18.59	5.81	5.80	2370	2370	50680	37%
23	23	18.54	18.56	5.84	5.83	2440	2405	53085	38%
24	24	18.53	18.54	5.86	5.85	2440	2440	55525	40%
25	25	18.54	18.54	5.87	5.87	2450	2445	57970	42%
26	26	18.5	18.52	5.89	5.88	2450	2450	60420	44%
27	27	18.45	18.48	5.92	5.91	2450	2450	62870	46%
28	28	18.45	18.45	5.94	5.93	2450	2450	65320	47%
29	29	18.44	18.45	5.96	5.95	2440	2445	67765	49%
30	30	18.44	18.44	5.97	5.97	2440	2440	70205	51%
			18.84		5.60		2338		
							2343		

Syringe#	Time	Animal#	tHb	SAT	HbCO	MetHb
1			13.7	93.4	0	0
			14.5	90.8	0	0
		AVE=>>>	14.1	92.1	0	0
2			14.2	86.8	0	0
			13.2	83.7	0	0
		AVE=>>>	13.7	85.25	0	0
3			13.8	91.4	26.3	0
			14.5	94.6	31.8	0
		AVE=>>>	14.15	93	29.05	0
5			13.7	86.1	59.7	0
			13.7	88.5	63.1	0
		AVE=>>>	13.7	87.3	61.4	0
4			17.4	20.9	65.8	0.3
			17.3	23.1	65.3	0
		AVE=>>>	17.35	22	65.55	0.15
6			14.6	88.5	64.5	0
			14.9	13.9	67.6	0.4
		AVE=>>>	14.75	51.2	66.05	0.2
"x"	animal 54		23.5	10.4	61.3	0
IC, died at 32 min..			20.8	4.4	60.5	0
		AVE=>>>	22.15	7.4	60.9	0

Syringe#	Tag#	Time	Type smp
1	50	0	art.
2	52	0	art.
3	50	5	art
4	52	20	IC

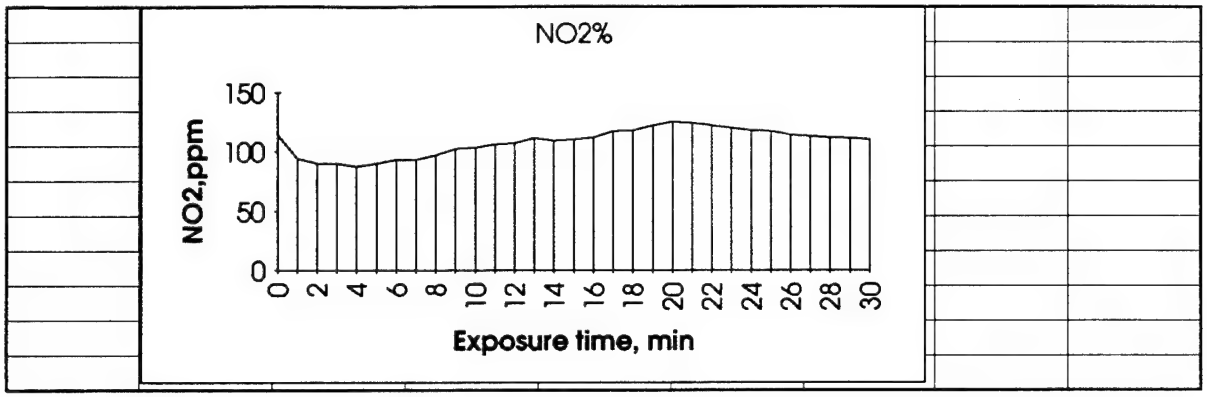
	5	50	15	art.				
	6	50	30	IC				

O2ct	HbO2	RHb	O2cap		pH	pCO2	pO2	HCO-
17.8	93.4	6.6	19		7.415	38.7	115	25.1
18.3	90.8	9.2	20.2		7.423	38.9	112	25.7
18.05	92.1	7.9	19.6		7.419	38.8	113.5	25.4
17.1	86.8	13.2	19.7		7.442	40.5	115	27.9
15.4	83.7	16.3	18.3					
16.25	85.25	14.75	19					
12.9	67.4	6.3	14.1		7.382	47.4	91	28.5
13	64.5	3.7	13.7		7.381	48.2	97	28.9
12.95	65.95	5	13.9		7.3815	47.8	94	28.7
6.6	34.7	5.6	7.7		7.133	52.6	78	17.8
6.2	32.7	4.2	7		7.123	53.5	91	17.9
6.4	33.7	4.9	7.35		7.128	53.05	84.5	17.85
1.7	7.1	26.8	8.2		6.891	64	8	12.4
1.9	8	26.7	8.3		6.89	64.2	15	12.4
1.8	7.55	26.75	8.25		6.8905	64.1	11.5	12.4
6.4	31.4	4.1	7.2		6.97	31.8	90	7.4
0.9	4.4	27.6	6.6		6.967	32.2	97	7.4
3.65	17.9	15.85	6.9		6.9685	32	93.5	7.4
1.3	4	34.7	12.6		6.49	87.3	4	6.7
0.5	1.7	37.8	11.4		6.491	86.1	4	6.6
0.9	2.85	36.25	12		6.4905	86.7	4	6.65

ANIMAL WEIGHTS		
tag#	grams	
47	379	
48	400	
49	395	
50	360	**control
51	383	
52	370	**control
53	389	
54	384	

TCO2	BEb	SBC	BEecf	%sO2c
26.2	1.2	25.9	0.3	98.6
26.9	1.9	26.4	1.2	98.5
26.55	1.55	26.15	0.75	98.55
29.2	4.1	28.1	3.6	98.6
29.9	3.2	27.4	3.2	96.8
30.4	3.5	27.6	3.6	97.3
30.15	3.35	27.5	3.4	97.05
19.4	-11	16.1	-11.5	90.2
19.5	-11	16.2	-11.5	93.5
19.45	-11	16.15	-11.5	91.85
14.4	-20.8	6	-20.8	3.3
14.4	-20.8	6.1	-20.8	7.7
14.4	-20.8	6.05	-20.8	5.5
8.4	-23	6.8	-24.5	90
8.4	-23	6.8	-24.6	91.9
8.4	-23	6.8	-24.55	90.95
9.4	-34.4	-4.7	-33	1
9.3	-34.4	-4.7	-33.1	0.9
9.35	-34.4	-4.7	-33.05	0.95

DATE:	120 NO2 & 5% CO2				27-28 September			
TIME	O2%	CO2%	CO,ppm	NO2%		Syringe#	Time	Animal#
0	19.26	5.55		114		1	0	82
1	19.33	4.89		94		1	0	82
2	19.3	4.92		90			0	82
3	19.13	5.24		90		2	0	86
4	19.02	5.33		87		2	0	86
5	18.96	5.24		90			0	86
6	18.88	5.27		93		3	5	82
7	18.8	5.3		93		3	5	82
8	18.79	5.25		97			5	82
9	18.78	5.17		102		4	10	86
10	18.74	5.18		103		4	10	86
11	18.66	5.12		106			10	86
12	18.66	5.08		107		5	15	82
13	18.59	5.07		111		5	15	82
14	18.56	5.11		109			15	82
15	18.53	5.11		110		6	30	86
16	18.52	5.05		112		6	30	86
17	18.48	4.98		117			30	86
18	18.47	4.97		118		7	60	82
19	18.41	4.93		122		7	60	82
20	18.41	4.89		125			60	82
21	18.38	4.9		124		8	120	86
22	18.36	4.92		122		8	120	86
23	18.31	4.91		120			120	86
24	18.3	4.96		118				
25	18.27	4.99		117			Time fr.	Freq, per
26	18.2	5.03		114			Zero time	minute
27	18.18	5.04		113			0	336
28	18.1	5.02		112			2.5	324
29	18.11	5.06		111			5	200
30	18.09	5.09		110			10	230
		5.082903		111			15	160
-				108.0968			20	148
ave==>	18.59935	5.082903	#DIV/0!	108.0968			25	144
							30	116

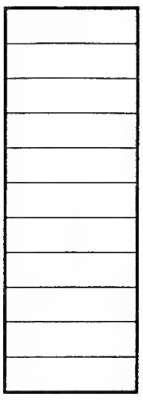

NO2%
NO2,ppm
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Exposure time, min

tHb	SAT	HbCO	MetHb	O2ct	HbO2	RHb	O2cap
8.5	93.9	0.6	0.7	11	92.7	6	11.7
8.5	94.4	0.6	0.6	11	93.3	5.5	11.7
8.5	94.15	0.6	0.65	11	93	5.75	11.7
15.9	91.6	0	0	20.2	91.6	8.4	22.1
15.1	88	0	0	18.5	88	12	21
15.5	89.8	0	0	19.35	89.8	10.2	21.55
8	89	0.6	1	9.7	87.6	10.8	10.9
7.9	88.7	0.6	1	9.6	87.3	11.1	10.8
7.95	88.85	0.6	1	9.65	87.45	10.95	10.85
13.7	82.4	0	0	15.7	82.4	17.6	19
14.1	84.1	0	0	16.5	84.1	15.9	19.6
13.9	83.25	0	0	16.1	83.25	16.75	19.3
8	92.7	0.6	1.7	10.1	90.6	7.1	10.9
7.6	94	0.9	1.4	9.7	91.8	5.9	10.3
7.8	93.35	0.75	1.55	9.9	91.2	6.5	10.6
14.4	73.4	0	1.7	14.4	72.1	26.2	19.7
14.2	74	0	1.7	14.3	72.7	25.6	19.4
14.3	73.7	0	1.7	14.35	72.4	25.9	19.55
7.5	85.8	1.2	1.3	8.7	83.7	13.8	10.2
7.4	86.3	0.9	1.6	8.7	84.1	13.4	10
7.45	86.05	1.05	1.45	8.7	83.9	13.6	10.1
14.5	2.5	0	0.4	0.5	2.5	97.1	20.1
14.5	2.6	0	0.3	0.5	2.6	97.1	20.1
14.5	2.55	0	0.35	0.5	2.55	97.1	20.1

>> 5	220

pH	pCO2	pO2	HCO-	TCO2	BEb	SBC	BEecf	%sO2c
7.225	36.7	121	15.3	16.5	-10.8	16.5	-12.5	97.9
7.236	37.9	124	16.3	17.4	-9.3	17.3	-11.4	98.1
7.2305	37.3	122.5	15.8	16.95	-10.05	16.9	-11.95	98
7.355	41.1	103	23.2	24.4	-1.7	23.6	-2.5	97.6
7.323	40.3	99	21.4	22.6	-3.7	22	-4.8	97.2
7.339	40.7	101	22.3	23.5	-2.7	22.8	-3.65	97.4
7.16	63.2	96	22.7	24.7	-6.4	19.8	-6.1	94.6
7.168	62	99	22.7	24.6	-6.3	20	-6	95.3
7.164	62.6	97.5	22.7	24.65	-6.35	19.9	-6.05	94.95
7.289	60.8	96	29.5	31.3	1.8	26.3	2.7	96.3
7.292	60.8	95	29.6	31.5	2	26.5	2.9	96.2
7.2905	60.8	95.5	29.55	31.4	1.9	26.4	2.8	96.25
7.216	62.3	100	25.5	27.4	-2.9	22.6	-2.4	95.6
7.223	62.6	98	26	28	-2.4	23	-1.8	95.8
7.2195	62.45	99	25.75	27.7	-2.65	22.8	-2.1	95.7
7.223	64.3	68	26.8	28.7	-1.8	23.3	-1.1	88.4
7.231	73.1	69	31	33.2	1.7	26	3.3	88.8
7.227	68.7	68.5	28.9	30.95	-0.05	24.65	1.1	88.6
7.291	53.7	73	26.2	27.8	-0.7	24.2	-0.6	92.3
7.294	54.4	76	26.6	28.3	-0.3	24.6	-0.1	93
7.2925	54.05	74.5	26.4	28.05	-0.5	24.4	-0.35	92.65
6.987	108	5	26.2	29.5	-7.6	16.2	-5.5	2.1
6.984	104.9	5	25.2	28.4	-8.4	15.6	-6.5	2
6.744	154	6	21.3	26	-17	8.9	-14.3	2.1
6.905	122.3	5.33	24.23	27.97	-11	13.57	-8.77	2.07

IC smp.
IC smp.
IC smp.
IC smp.



14-Oct	2600 CO, 111 NO2							
time,min	O2%	CO2%	CO,ppm	NO2,ppm	NO2,(c)	NO2, k	%,LC k	CO, k
0	20.1	0.58	2340	161	94	94	1.57	2340
1	19.96	0.76	2280	157	90	184	3.07	4620
2	19.93	0.72	2280	156	89	273	4.55	6900
3	19.85	0.78	2480	158	91	364	6.07	9380
4	19.82	0.8	2530	163	96	460	7.67	11910
5	19.78	0.81	2580	168	101	561	9.35	14490
6	19.74	0.85	2670	171	104	665	11.08	17160
7	19.68	0.85	2620	175	108	773	12.88	19780
8	19.61	0.92	2650	180	113	886	14.77	22430
9	19.55	0.94	2580	186	119	1005	16.75	25010
10	19.54	0.96	2620	190	123	1128	18.80	27630
11	19.49	0.99	269	190	123	1251	20.85	27899
12	19.42	1.09	2740	190	123	1374	22.90	30639
13	19.39	1.14	2720	186	119	1493	24.88	33359
14	19.32	1.21	2740	185	118	1611	26.85	36099
15	19.3	1.27	2930	180	113	1724	28.73	39029
16	19.27	1.31	2950	178	111	1835	30.58	41979
17	19.26	1.32	2970	177	110	1945	32.42	44949
18	19.25	1.34	3000	175	108	2053	34.22	47949
19	19.2	1.34	2990	173	106	2159	35.98	50939
20	19.18	1.42	2970	174	107	2266	37.77	53909
21	19.19	1.4	2910	176	109	2375	39.58	56819
22	19.18	1.4	2870	179	112	2487	41.45	59689
23	19.18	1.39	2840	184	117	2604	43.40	62529
24	19.16	1.41	2800	186	119	2723	45.38	65329
25	19.15	1.42	2760	188	121	2844	47.40	68089
26	19.15	1.42	2710	190	123	2967	49.45	70799
27	19.15	1.41	2700	189	122	3089	51.48	73499
28	19.15	1.41	2700	187	120	3209	53.48	76199
29	19.15	1.41	2700	187	120	3329	55.48	78899
30	19.15	1.41	2700	188	121	3450	57.50	81599
	19.43	1.14	2632.226		111.2903	1879.643		
			Note: animals were still alive at 26 minutes into exposure, four					

% LC k CO		Syringe#	Time	Animal#	tHb	SAT	HbCO	MetHb
1.70		1	0	108	14.4	90.6	0	0
3.35					14.5	91.6	0	0
5.00				AVE>>>	14.45	91.1	0	0
6.80								
8.63		2	0	110	11.7	92	0	0.3
10.50					11.7	92.2	0	0.5
12.43				AVE>>>	11.7	92.1	0	0.4
14.33								
16.25		3	5	108	15.1	95.1	21.9	0.5
18.12					14.9	94.7	22	0.6
20.02				AVE>>>	15	94.9	21.95	0.55
20.22								
22.20		4	10	110	10.3	89.8	56.9	0
24.17					10.2	89.3	55.8	0
26.16				AVE>>>	10.25	89.55	56.35	0
28.28								
30.42		5	15	108	11.7	69.9	43.8	0
32.57					12.3	71.1	47.8	0
34.75				AVE>>>	12	70.5	45.8	0
36.91								
39.06		NA	20	110	10.4	27	73.3	2.7
41.17		rat removed from chamber			10.4	29.6	73.2	2.81
43.25		at 20 min for CP		AVE>>>	10.4	28.3	73.25	2.755
45.31		("almost dead already)						
47.34		NA	30	108	14.9	6.4	73	1.6
49.34		rat removed at 30 min			15	6.2	72.8	1.4
51.30		for CP, dead		AVE>>>	14.95	6.3	72.9	1.5
53.26								
55.22		NA	30	106	16.1	5.5	73.5	1.9
57.17		rat dead at end of exp. CP			16	2.6	73.6	0.9
59.13		doen for blood		AVE>>>	16.05	4.05	73.55	1.4
were dead when removed from restrainers ...just post exp(1 died at 27.5 min)								

O2ct	HbO2	RHb	O2cap	pH	pCO2	pO2	HCO-	TCO2
18.1	90.6	9.4	20	7.398	43.2	89	26.9	28.2
18.5	91.9	8.1	20.2	7.405	43.7	91	27.7	29.1
18.3	91.25	8.75	20.1	7.4015	43.45	90	27.3	28.65
14.9	91.7	8	16.2	7.38	43.9	84	26.2	27.6
14.9	91.8	7.7	16.2	7.386	43.9	96	26.6	28
14.9	91.75	7.85	16.2	7.383	43.9	90	26.4	27.8
15.5	73.8	3.8	16.3	7.372	52.1	71	30.6	32.2
15.2	73.3	4.1	16	7.367	51.3	72	29.7	31.3
15.35	73.55	3.95	16.15	7.3695	51.7	71.5	30.15	31.75
5.5	38.7	4.4	6.2	7.225	38.3	82	16	17.2
5.6	39.5	4.7	6.3	7.226	36.9	83	15.5	16.6
5.55	39.1	4.55	6.25	7.2255	37.6	82.5	15.75	16.9
6.4	39.3	16.9	9.1	7.109	37	47	11.8	13
6.3	37.1	15.1	8.9	7.1	38	56	11.9	13.1
6.35	38.2	16	9	7.1045	37.5	51.5	11.85	13.05
0.9	6.5	17.5	3.5	6.609	74.5	10	7.5	9.8
1	7.1	16.9	3.5	6.606	74.9	10	7.5	9.8
0.95	6.8	17.2	3.5	6.6075	74.7	10	7.5	9.8
0.3	1.6	23.8	5.3	6.654	82.9	3	9.3	11.9
0.3	1.6	24.2	5.4	6.656	81.9	3	9.2	11.7
0.3	1.6	24	5.35	6.655	82.4	3	9.25	11.8
0.3	1.3	23.3	5.5	6.664	83.4	1	9.6	12.1
0.2	0.7	24.8	5.7	6.67	80.6	1	9.4	11.9
0.25	1	24.05	5.6	6.667	82	1	9.5	12

BEb	SBC	BEecf	%sO2c
2.3	26.7	1.9	96.8
3.1	27.3	2.8	97
2.7	27	2.35	96.9
1.3	25.9	0.9	96
1.8	26.3	1.4	97.3
1.55	26.1	1.15	96.65
4.6	28.4	5.1	93.3
3.8	27.8	4.2	93.4
4.2	28.1	4.65	93.35
-10.3	16.8	-11.8	93.7
-10.7	16.5	-12.3	94
-10.5	16.65	-12.05	93.85
-16.3	11.4	-17.9	68.7
-16.4	11.5	-17.9	77
-16.35	11.45	-17.9	72.85
-31	2	-30.3	3.2
-31.1	-2.1	-30.3	3
-31.05	-0.05	-30.3	3.1
-28.6	-0.2	-27.7	1
-28.6	-0.2	-27.8	0.9
-28.6	-0.2	-27.75	0.95
-28.1	0.1	-27.3	0.2
-28.1	0.1	-27.4	0.5
-28.1	0.1	-27.35	0.35

time,min	O2%	CO2%	CO,ppm	NO2,ppm	NO2,(c)	NO2, k	%,LC k	CO, k
0	19.56	4.3	1660	194	94	94	1.57%	1660
1	19.94	4.45	1670	191	91	185	3.08%	3330
2	19.31	4.52	1700	187	87	272	4.53%	5030
3	19.23	4.63	1760	186	86	358	5.97%	6790
4	19.18	4.62	1760	189	89	447	7.45%	8550
5	19.09	4.66	1750	191	91	538	8.97%	10300
6	19.06	4.64	1840	192	92	630	10.50%	12140
7	19.04	4.64	1990	192	92	722	12.03%	14130
8	18.98	4.66	1980	192	92	814	13.57%	16110
9	18.9	4.69	1960	192	92	906	15.10%	18070
10	18.9	4.66	1960	194	94	1000	16.67%	20030
11	18.87	4.62	2050	197	97	1097	18.28%	22080
12	18.85	4.52	2030	204	104	1201	20.02%	24110
13	18.81	4.51	2020	207	107	1308	21.80%	26130
14	18.8	4.49	1960	212	112	1420	23.67%	28090
15	18.71	4.5	1910	211	111	1531	25.52%	30000
16	18.73	4.36	2060	219	119	1650	27.50%	32060
17	18.69	4.42	2040	219	119	1769	29.48%	34100
18	18.65	4.42	2050	219	119	1888	31.47%	36150
19	18.64	4.45	2030	220	120	2008	33.47%	38180
20	18.59	4.47	2010	220	120	2128	35.47%	40190
21	18.54	4.52	1970	218	118	2246	37.43%	42160
22	18.53	4.5	1970	221	121	2367	39.45%	44130
23	18.53	4.48	2010	222	122	2489	41.48%	46140
24	18.51	4.45	2020	223	123	2612	43.53%	48160
25	18.53	4.41	2010	224	124	2736	45.60%	50170
26	18.51	4.43	2010	223	123	2859	47.65%	52180
27	18.49	4.44	2000	222	122	2981	49.68%	54180
28	18.48	4.46	2000	219	119	3100	51.67%	56180
29	18.47	4.47	1990	217	117	3217	53.62%	58170
30	18.46	4.46	1990	216	116	3333	55.55%	60160

	Column 1	*O2%*		*Column 1*	*CO2%*
	Mean	18.82516		Mean	4.51129
	Standard	0.063169		Standard	0.017875
	Median	18.73		Median	4.49
	Mode	18.53		Mode	4.45
	Standard	0.351709		Standard	0.099524
	Variance	0.123699		Variance	0.009905
	Kurtosis	2.158287		Kurtosis	-0.66541
	Skewness	1.383064		Skewness	0.237904
	Range	1.48		Range	0.39
	Minimum	18.46		Minimum	4.3
	Maximum	19.94		Maximum	4.69
	Sum	583.58		Sum	139.85

	Count	31		Count	31			
	Column 1	*CO, ppm*		*Column 1*	*NO2, ppm*			
	Mean	1940.645		Mean	207.5161			
	Std error	21.68164		Standard	2.479972			
	Median	1990		Median	212			
	Mode	2010		Mode	192			
	Std Dev	120.7183		Standard	13.8079			
	Variance	14572.9		Variance	190.6581			
	Kurtosis	0.396672		Kurtosis	-1.73127			
	Skewness	-1.32996		Skewness	-0.29659			
	Range	400		Range	38			
	Minimum	1660		Minimum	186			
	Maximum	2060		Maximum	224			
	Sum	60160		Sum	6433			
	Count	31		Count	31			

% LC k CO	LC% sum			Syringe#	Time	Animal#	tHb	SAT
1.20%	2.77%			2	0	117	15	78.4
2.41%	5.50%						15.5	79.9
3.64%	8.18%						15.25	79.15
4.92%	10.89%							
6.20%	13.65%			4	5	117	14	85.6
7.46%	16.43%						14.3	87.6
8.80%	19.30%						14.15	86.6
10.24%	22.27%							
11.67%	25.24%			6	15	117	13.1	86
13.09%	28.19%						13.5	86.4
14.51%	31.18%						13.3	86.2
16.00%	34.28%							
17.47%	37.49%				20	113	14.8	8.6
18.93%	40.73%				animal removed &		14.7	9.6
20.36%	44.02%				sample taken IC*		14.75	9.1
21.74%	47.26%							
23.23%	50.73%				30	117	13.5	18.3
24.71%	54.19%				animal removed &		13.3	11.6
26.20%	57.66%				sample taken IC*		13.4	14.95
27.67%	61.13%							
29.12%	64.59%				30+	112	16.3	5.2
30.55%	67.98%				** (IC sample)		16.1	4.5
31.98%	71.43%						16.2	4.85
33.43%	74.92%							
34.90%	78.43%				90 min Post-exp			
36.36%	81.96%				***	#119	20.7	2.8
37.81%	85.46%				sample taken IC		20.9	3.6
39.26%	88.94%						20.8	3.2
40.71%	92.38%							
42.15%	95.77%							
43.59%	99.14%				* -- animal was euthanized just prior to sc			
					** -- animal died just post exposure & wa			
					*** -- animal died at 90 minutes post-exp			

HbCO	MetHb	O2ct	HbO2	RHb	O2cap	pH	pCO2	pO2
0	0	16.3	78.4	21.6	20.9	7.344	52.4	75
0	0	17.2	79.9	20.1	21.5	7.347	52.2	75
0	0	16.75	79.15	20.85	21.2	7.3455	52.3	75
8.5	0	15.2	78.3	13.2	17.8	7.274	63.7	89
13.7	0	15	75.6	10.7	17.2	7.277	62.7	88
11.1	0	15.1	76.95	11.95	17.5	7.2755	63.2	88.5
41.2	0	9.2	50.6	8.2	10.7	7.277	62.3	82
42	0	9.4	50.1	7.9	10.9	7.274	61	86
41.6	0	9.3	50.35	8.05	10.8	7.2755	61.65	84
62.9	0.9	0.6	3.1	33.1	7.4	6.994	93.4	4
63	0.8	0.7	3.5	32.7	7.4	6.994	90.7	4
62.95	0.85	0.65	3.3	32.9	7.4	6.994	92.05	4
68.3	1.4	1.1	5.6	24.7	5.7	6.837	79	9
68.5	1.3	0.6	3.5	26.7	5.6	6.833	77.9	12
68.4	1.35	0.85	4.55	25.7	5.65	6.835	78.45	10.5
65.9	2.5	0.4	1.7	29.9	7.2	6.657	96.6	4
66	2.3	0.3	1.4	30.3	7.1	6.655	76	5
65.95	2.4	0.35	1.55	30.1	7.15	6.656	86.3	4.5
9.8	0.3	0.7	2.5	87.4	25.9	6.836	110.7	4
9.9	0.3	0.9	3.2	86.6	26.1	6.844	109.9	7
9.85	0.3	0.8	2.85	87	26	6.84	110.3	5.5

mpling
s then sampled
& was sampled immediately

HCO-	TCO2	BEb	SBC	BEecf	%sO2c			
28.8	30.4	2.6	26.8	2.9	93.8			
28.9	30.5	2.7	26.9	3	93.8			
28.85	30.45	2.65	26.85	2.95	93.8			
29.8	31.7	1.7	26.2	2.7	95.1			
29.6	31.5	1.7	26.1	2.6	95.1			
29.7	31.6	1.7	26.15	2.65	95.1			
29.4	31.3	1.5	26	2.4	94	"error pO2 end pt"		
28.5	30.4	0.7	25.4	1.5	94.6			
28.95	30.85	1.1	25.7	1.95	94.3			
22.9	25.8	-10	14.3	-8.6	1.9	"analyzer ??'d low O2%"		
22.3	25.1	-10.6	13.9	-9.3	1.8			
22.6	25.45	-10.3	14.1	-8.95	1.85			
13.5	15.9	-21.1	5.7	-20.6	3.7	"analyzer ??'d low O2%"		
13.2	15.6	-21.4	5.5	-20.9	5			
13.35	15.75	-21.25	5.6	-20.75	4.35			
10.9	13.9	-27.2	0.9	-26.1	1.1	"analyzer ??'d low O2%"		
8.6	10.9	-29.2	-0.6	-28.5	1.4			
9.75	12.4	-28.2	0.15	-27.3	1.25			
18.9	22.3	-16.8	9	-15.2	1.4	"analyzer ??'d low O2%"		
19.1	22.5	-16.5	9.3	-14.9	2.5			
19	22.4	-16.65	9.15	-15.05	1.95			

Addendum

To meet the military objective of determining a criteria for incapacitation and lethality from toxic gas exposure, a series of small animal tests and data analyses were conducted. A narcotic gas (CO) and an irritant gas (NO2), along with carbon dioxide (CO2), were tested in all combinations: CO, NO2, CO2, CO + CO2, NO2 + CO2, and NO2 + CO + CO2. A group of six animals was exposed to each gas combination and lethality and biophysical data was collected.

We conclude that our observations of lethality from single toxic gasses can be correlated with a fractional effective dose description, in which external concentrations are corrected for minute volume changes. Multiple gas exposures clearly demonstrate synergistic effects because lethality rates greatly exceed those expected from statistically independent causes. Simple addition of the FED values, however, overstates the effect and implies a competition between the narcotic and irritant gas effects. The N-Gas model, while being an additive FED model, does not appear to be in a form that could guide the setting of military exposure standards.

Background

The survivability of armored combat vehicles, ACVs, depends on the vulnerability of both the vehicle and the crew. Until a few years ago, evaluation of the vehicle vulnerability was limited to an assessment of the armor's ability to prevent penetration by a specified anti-armor threat and an evaluation of the vehicle systems and components through selective engineering tests. Questions arose regarding the accuracy of this method, which prompted the Office of the Secretary of Defense to initiate the Joint Live Fire Program in 1984. Congress then passed Live Fire Test legislation in 1987 to require live fire testing of all United States weapon platforms against realistic combat threats.

"Behind-armor" events produce a number of potential hazards to the crew including fragment injury, blast overpressure, and exposure to toxic gases produced by burning materials or the penetrating object (Mayorga, 1994). Improvements in design and protective gear have greatly enhanced the survivability of the crew in recent years. The crew in a present day ACV incident is more likely to survive, to remain in the vehicle and to continue the combat mission. This likelihood increases the importance of accurately evaluating crew injury or incapacitation. In response, the Army Medical Department is evaluating nonfragment injuries for live fire testing, identifying potential hazards, and standardizing instrumentation requirements.

Criteria have been established capable of predicting injury due to blast overpressure, thermal exposure, acceleration loads, and toxic gases in less hostile environments. For example, in the area of toxic gases, most medical studies reported in the literature focus on low level chronic exposures typical of environmental exposures to air

pollutants. In order to confidently assess injury potential under battlefield conditions requires investigation through medical research specifically designed to characterize the hazards in these environments.

The Department of Respiratory Research at the Walter Reed Army Institute of Research (WRAIR) has funded and conducted research on the effects of brief exposures to high levels of nitrogen dioxide, probably the least characterized of the potential toxic combustion gases found in battlefield scenarios. This work has involved both large and small animal models. This work has resulted in guidance criteria in terms of an incapacitating dose for each toxic gas component and a reduction factor for the presence of carbon dioxide.

The Federal Aviation Administration (FAA) has evaluated the toxicity of atmospheres generated in typical fires on board aircraft to determine minimum survival times for escape and maximizing the time to flashover. They have proposed separate correlations for narcotic and irritant gasses, each taking into account the effects of CO2 on minute volumes.

The Fire Safety Department at the National Institute of Standards & Technology (NIST), formerly the National Bureau of Standards has also had an active research program. NIST researchers and the fire safety & commercial industries have evaluated various methods for determining the toxic potency of burning materials by looking at lethality levels in small animal exposures. This work has produced a single correlation of lethality using both irritant and narcotic gas concentrations, known as the N-Gas equation. The effects of CO2 are included as an amplification of the CO toxicity. NO2 effects are not included.

To meet the military objective of determining a criteria for incapacitation and lethality from toxic gas exposure a series of small animal tests and data analyses were conducted. A narcotic gas (CO) and an irritant gas (NO2), along with carbon dioxide (CO2), were tested in all combinations: CO, NO2, CO2, CO + CO2, NO2 + CO2, and NO2 + CO + CO2. was collected. A group of six animals was exposed to each gas combination and lethality and biophysical data was collected. This data was used to evaluate the accuracy of the N-Gas model, with a fractional dose for nitrogen dioxide added, and to explore other mathematical formulations.

Methodology

Mature male Sprague Dawley rats weighing 250 - 350 grams (at time of exposure) were procured from Charles River Laboratory, Boston MA, and placed in quarantine in accordance with WRAIR standard operating procedures. After delivery from quarantine, animals were randomized, segregated into groups of eight animals per group, identified by ear tags, housed in individual micro-isolators, and allowed to acclimate for one week prior to exposure. Animals were weighed at least every two days and monitored for normal growth.

On the day before the planned exposure, two animals were selected from the group for catheter placement. The catheter (a six inch length of PE50 tubing) was placed in the caudal tail artery and sutured in placed. The animal was placed in a jacket collar which allowed movement but prevented access to the tail area and returned to the cage for the night.

The exposure chamber consisted of a 134 liter Plexiglas box, about 4 feet long, 12 inches wide and 16 inches high. Reagent grade gases were combined at atmospheric pressure in a four-channel stainless steel mixer to the pre-selected composition and delivered to the inlet port on the chamber (Figure 1) via 0.250"ID Teflon tubing.

The composition of the atmosphere inside the chamber was constantly monitored by gas analyzers. Individual pumps on the analyzers drew air samples through a six-port sampling manifold made of non-reactive plastics and into the appropriate analyzer cell. After analysis, the air was returned to the chamber via return ports on the top of the chamber. Excess air flow in the sampling manifold was returned to the chamber via a

return inlet on the bottom of the chamber. This arrangement maintained constant mixing while simulating the closed atmospheres typical of an explosion and fire in an armored vehicle.

The following gases [in the exposure chamber atmosphere] were monitored: % oxygen by electrochemical analyzer (Ametek model S3A-1, Thermox Instruments, Pittsburgh, PA); % carbon dioxide by infrared analyzer (Ametek model CD-3A, Thermox Instruments, Pittsburgh, PA); carbon monoxide, ppm levels, by nondispersive infrared-ultraviolet (IR-UV) spectrophotometer, (Binos, Inficon Leybold-Heraeus, Federal Republic of Germany); and nitrogen dioxide, ppm levels, by dual-beam infrared-ultraviolet (IR-UV) spectrophotometer (Binos, Inficon Leybold-Heraeus, Inc., Federal Republic of Germany). Atmospheres were also sampled for contamination by nitrogen monoxide (NO) using dual-beam infrared-ultraviolet (IR-UV) spectrophotometer (Binos, Inficon Leybold-Heraeus, Inc., Federal Republic of Germany). NO was not detected as a contaminate in any of the exposures.

The exposure chamber contained an 8-liter inner chamber which was gasketed from the exposure atmosphere. This inner box contained the inlet portholes into which the animal restrainers were placed. Medical grade breathing air was pumped through this chamber while the animals were being loading into the chamber prior to exposure. The chamber was fitted with a remotely operated door which was released, dropping to the floor of the chamber and allowing the test atmosphere to contact the animals. The thirty minute exposure time started at the moment the door was dropped. The test atmosphere as monitored by the various analyzers equilibrated in less than 90 seconds.

The animals to be exposed were placed into Lexan restrainers [Part number 70054 sleeve fitted with part number 70057 stainless steel tail tube/back plate, Lab Products, Inc., Rockville, MD]. See Figure 2. These restrainers are designed for use in flow-through nose-only exposure chamber. The animal was placed in the tube and his tail was fed into the tail tube; the metal tail tube acted as a heat sink for the tail to moderate increases in body temperature during the prolonged restraint required for these inhalation exposure studies. The back plate was then adjusted snugly against the animal's rump, positioning the nose firmly into the inlet port. The animals were placed into the restrainers as quickly as possible to minimize stress and loaded into the exposure chamber at once. As noted above, the animals were exposed only to medical grade air, flowing at 12 to 18 cubic feet per minute, until the exposure door was opened.

Eight animals were exposed in each test. No animal was exposed more than once. Additional sets of animals were run as controls, breathing only medical grade air or medical grade air containing 5% carbon dioxide. As noted above, two of the animals had been fitted the previous day with catheters in the caudal tail artery. Those two animals were placed in tube restrainers which had been modified to allow access to the tail. Pre-exposure (or "zero time") blood samples were drawn from each animal just prior to being loading into the chamber for analysis. Additional samples were drawn at 5 minutes into the exposure, 10 minutes into the exposure, 15 minutes into the exposure, at 30 minutes (the end of the exposure), at 30 minutes post-exposure and at 90 minutes post exposure. Samples were drawn alternately from the two catheterized animals so that one animal was sampled at 0, 5, 15 and 30 minutes, while the other was sampled at 0, 10, 30 and 90 minutes post exposure. Marquest part number 601 "Aspirator" blood gas syringes containing 50 units of dry lithium heparin were used for all blood samples [Marquest

Medical Products, Englewood, CO]. At least 0.7 mls of blood was drawn to minimize concentration effects from the heparin.

All samples were analyzed as quickly as possible for pH & blood gases (Instrumentation Laboratories IL1306 analyzer) and hemoglobin levels (Radiometer-Copenhagen OSM3 Hemoximeter).

In addition, three of the remaining six animals were selected at random and placed in restrainers which had been modified with adapters designed to receive a linear pneumotach [Model 8411B non-heated pneumotachometer, Hans Rudolph, Kansas City, MO]. The modification of the restrainer allowed it to be used as a partial body phythesmography enclosure - the deflections of the chest wall during respiration were recorded as changes in the pressure across the screens in the pneumotach housing. The inlet and outlet ports on the pneumotachs were connected to low range differential pressure transducers [MP45-20-817, Validyne Engineering Corporation, Northridge, CA]. Pressure flow data was collected at the rate of 40 samples/second/channel and recorded using a computerized data acquisition system [DATAQ Instruments, Akron, OH]. Complete respiratory curves were recorded for the three animals throughout the thirty minutes exposure duration. Respiration rate and time of death were obtained from these recordings. The curves were integrated and compressed to yield inspired minute volume, expired minute volume, tidal volume, and delta [V], the difference in inspired and expired volume.

Lung weights were done on selected animals, both in controls and in exposures involving NO2, either alone in air or combined with carbon monoxide. Increases in the ratio of "wet" lung weight to dry lung weight is a qualitative indicator of pulmonary edema or damage from irritant inhalants. Immediately after euthanasia, the chest cavity of the catheterized animals was opened and the lungs removed. The lobes were separated,

perfused with saline, blotted on paper toweling and placed in prepared petri dishes. The lungs were weighed and then placed into an oven at 36C and re-weighed every 12 hours to constant weight.

At the conclusion of the thirty minute exposure time, all eight animals were quickly removed from the chamber and returned to normal air. The non-catheterized animals were removed from the restrainers, visually assessed for physical condition and returned to individual cages in the laboratory. The animals were closely monitored for two hours following exposures as it was noted that deaths typically occurred within or shortly after exposure.

At the end of the two hour post-exposure observation period, the animals were returned to their microisolator cages in the animal room. Body weights were recorded daily for at least 14 days following the exposures; in some cases, weights were tracked for 30 or 45 days.

After final blood samples were collected from the two catheterized animals, the animals were lightly anethesized with Ketamine/Rompun (0.1 ml per 100 g) and euthanized in a CO2 chamber. After euthanasia, the lungs were removed, if appropriate to the exposure protocol. During exposures involving NO2, the lungs of any animals which died during or post-exposure were also removed for wet/dry lung weight analysis.

Results

Table 1 summarizes the exposures conducted and the lethality results. These combinations were selected to validate LC50 values reported by other researchers and in prior WRAIR-supported NO2 work. It is recognized that simple binomial expansion probability suggests that the results obtained are not statistically valid, but, since the intent was merely to double-check previously reported LC50 values in adult male rats, duplicate exposures were not conducted. No deaths in any of the exposures occurred more than 24 hours post exposure.

In order to correlate these results with FED, we first select a correction for minute volume changes with CO2 concentration. Figure 3 shows the measured average minute volumes variation with CO2 concentration, divided into three groups: those animal exposed to CO, NO2, and CO + NO2. First, a minute volume at 0% CO2 was estimated. Then an exponential least squares regression was made between minute volume and the CO2 concentration. Although the amount of data is limited, the effect of CO2 clearly depend upon the toxic gas present. In the presence of CO, minute ventilation is 2.27 times greater at 5% CO2 than the value estimated at 0%. With NO2 present, the minute volume increases by only 1.41 times and with both CO and NO2 present, an intermediate increase of 1.74 is observed. The data suggests that NO2 has a depressing effect on minute volume, at least for this species.

There is not enough data to develop reliable correlations of minute volume changes with all gas species, so we adopt the mean exponential regression for the minute volume multiplier

$$V_{CO2} = \exp(0.1108\,[CO_2\,\%])$$

to be used in the fraction effective dose relation

$$FED(X) = V_{CO2}\,\frac{[X]}{LC50(X)}$$

Using this correction to extrapolate results to 0% CO2, LC50 values of 200 ppm and 4500 ppm were determined for NO2 and CO, respectively, for the limited data collected in this experiment.

The dichotomous nature of the end point, death, suggests the log-logit correlation

$$\ln\left(\frac{p}{1-p}\right) = a + b\ln(FED)$$

where p is the probability of lethality, or lethality fraction, and a and b are coefficients of the regression. Since the FED is designed to have a value of 1 at 50% lethality, then $a = 0$. If we define the normalized FED value as

$$f = FED^b$$

then p takes the simple form

$$p(f) = \frac{f}{1+f}$$

The open symbols in Figure 4 show that the lethality fraction from single toxic gas exposures can be correlated with the minute volume corrected fractional dose using the logistic regression.

We now consider the experiments in which both narcotic and irritant gasses were present. If the lethalities due to each gas are statistically independent, then the probability of injury would be given by

$$\begin{aligned} p(f_1, f_2) &= p(f_1) + p(f_2) - p(f_1)\, p(f_2) \\ &= p(f_1 + f_2 + f_1\, f_2) \end{aligned}$$

The closed symbols in Figure 4 show the combined gas cases. Clearly, the two modes of lethality are not statistically independent and there must be a synergistic coupling.

The other extreme of assumptions is that processes have identical biological pathways and the FEDs can be simply added. The results of this assumption are shown on Figure 5. Clearly, the lethality of combined gas exposure is now overestimated, suggesting that the coupling of the lethality modes is not perfect. This result is not surprising, considering the physiological pathways for narcotic and irritant gasses.

A third assumption is that the two processes compete with each other through an intermediate resulting in the rule for addition

$$FED = \frac{FED_1 + FED_2}{1 + FED_1\, FED_2}$$

Application of this rule leads to the correlation shown in Figure 6, which produces somewhat better agreement. The same correlation expressed in terms of the FED itself is shown in Figure 7.

Discussion

The limited number of tests can only suggest a qualitative trend and because only single groups of animals were exposed at each condition, the quantitative values have an unknown range of variation. The LC50(NO2) value of 200 ppm is not very different from that reported by other researchers, but the LC50(CO) value of 4500 ppm is somewhat lower. Some variation can be expected between species, source of test animals, and even test conditions. The scaling of results by the observed LC50 values is important in producing comparable results.

The exponential variation of minute volume with CO2 concentration has been suggested by Purser (1985). A coefficient that produces a factor of 2.2 increase in rats at 5% CO2 for rats, suggested by Speitel (1994), agrees well with the factor of 2.27 found in the current study.

The surprising result that NO2 decreases minute volume is supported by previous studies. Lehnert , et al. (1994) report that the CO2-associated increases averaged only about 40% during 100-300 ppm NO2 + 5% CO2 exposures. This finding agrees closely with the increase of 41% found in this study. As a cautionary note, the Lehnert study found that the effect varied with NO2 concentration and time of exposure and affects breathing frequency and tidal volume differently.

This study selected an average minute volume correction factor that produced a factor of 1.74 increase in effective dose in the presence of 5% CO2. This value is significantly less, however, than the observed minute volume change, a factor of 2.27, in the presence of CO, yet it provides a good correlation with CO-induced lethality. Speitel

(1994) observes that COHb formation in rats (before saturation) was 1.5 times greater for 2500 ppm CO in the presence of 5.25% CO2 than in its absence and that the apparent uptake increase (based on LC50 data) ranged from 1.2 to 1.6 fold, even though the observed minute volume increase was 2.2. Therefore, there is support for the choice of effective dose multiplier for CO in the presence of CO2 (although the explanations is more complicated).

The effective dose relation suggested by Levin (1987) for the effect of CO2 on the lethality of CO is of the form

$$\frac{m[CO]}{[CO_2]-b}$$

where m = -18 and b= 122,000 ppm, which can be written as

$$V_{CO2}\,\frac{[CO]}{LC50(CO)}$$

where

$$V_{CO2} = \frac{1}{1-\frac{[CO_2]}{b}} = \frac{1}{1-\frac{[CO_2]}{122{,}000}}$$

and

$$LC50(CO) = -\frac{b}{m} = 6777\ ppm.$$

The effective dose multiplier at 5% CO2 is 1.69, which is again close to the effective value of 1.74 used in this study.

The N-Gas model reported by Levin (1987) included the effects of narcotic and irritant gasses as a sum of fractional effective doses. Only the FED for CO was corrected for the increase in minute volume and the effects of NO2 were not included. Following the

same pattern, we generalize the N-gas model by adding the fractional dose for NO2 without CO2 correction. Levin (1994) has taken a similar approach. The extended N-Gas model, without the terms for gasses not present, would be

$$N-Gas\ Value = \frac{m[CO]}{[CO_2]-b} + \frac{21-[O_2]}{21-LC50(O_2)} + \frac{[NO_2]}{LC50(NO_2)}$$

The comparison of the N-Gas value with observed lethality fraction is shown in Figure 8. The model significantly underpredicts lethality from the single toxic gas exposures. The underprediction of the CO tests arises from the lower value of LC50(CO) observed in these tests, 4500 ppm, compared with the 6777 ppm value implied by the N-Gas equation. If the LC50 value could be added explicitly, these points could be brought into agreement, but the combined gas exposures would then be overpredicted. The underprediction of the NO2 + CO2 exposure arises from the lack of a minute volume correction in the NO2 term.

We conclude that our observations of lethality from single toxic gasses can be correlated with a fractional effective dose description, in which external concentrations are corrected for minute volume changes. Multiple gas exposures clearly demonstrate synergistic effects because lethality rates greatly exceed those expected from statistically independent causes. Simple addition of the FED values, however, overstates the effect and implies a competition between the narcotic and irritant gas effects. The N-Gas model, while being an additive FED model, does not appear to be in a form that could guide the setting of military exposure standards.

Progress in the following areas would provided additional insight into the problem. First, the effective uptake is less than the minute volume increase, implying a changing

physiological situation that may be related to tidal volume or residence time. Second, the overestimation of effect by simple addition implies an antagonism between the narcotic and irritant gasses that may be very important in very short duration exposures. Finally, species differences, both in physical scale and physiological response, are so broad that these concepts must be translated to a more satisfying physiological basis so that extrapolation to man can be made with confidence.

ACKNOWLEDGMENTS

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REFERENCES

Lehnert, B.E., Archuleta, D.C., Ellis, T., Session, W.S., Lehnert, N.M., Gurley, L.R., and Stavert, D.M. 1994 Lung injury following exposure of rats to relatively high mass concentrations of nitrogen dioxide. *Toxicology*, **89**, 239-277.

Levin, B.C., Braun, E., Navarro, M., and Paabo, M. 1994. Further developments of the N-Gas model: effects of NO2 induced injury. Report for Contact MIPR41720023, NIST, Gaithersburg, MD.

Levin, B.C., Paabo, M., Gurman, J.L., and Harris, S.E. 1987. Effect of exposure to single or multiple combinations of the predominant toxic gasses and low oxygen atmospheres produced by fires. *Fundam. Appl. Toxicol.*, **9**, 236-250.

Mayorga, M.A. 1994. Overview of nitrogen dioxide effects on the lung with emphasis on military relevance. *Toxicology*, **89**, 175-192.

Purser, D.A. 1985. Toxicity assessment of combustion products. In **SFPE Handbook of Fire Protection Engineering**, ed. DiNenno, P.J., National Fire Protection Association, Quincy, MA.

Ripple, G.R. and Mundie, T.G. 1989. **Medical Evaluation of Nonfrangment Injury Effects in Armored Vehicle Live Fire Tests. Instrumentation Requirements and Injury Criteria**. Report of Department of Respiratory Research, Division of Medicine, Walter Reed Army Institute of Research, Washington, D.C.

Speitel, L.C. 1994. Fractional effective dose model for postcrash aircraft survivability. In **Proceedings of Fifth Annual BBC Conference on Flame Retardancy:**

Recent Advances in Flame Retardancy of Polymeric Materials, Stamford, CT.

EXPOSURE CONDITIONS & RESULTS			AVERAGE GAS CONCENTRATION			
Test date	Exposure Conditions	Results	O2%	CO2 ppm	NO2 ppm	CO ppm
25-Aug	AIR CONTROLS	0/6 DIED	20.4	5100	0	0
30-Aug	5% CO2 CONTROLS	0/6 DIED	19.5	52200	0	0
1-Sep	2000 CO IN AIR	0/6 DIED	19.5	12800	0	2010
7-Sep	4500 CO IN AIR	4/6 DIED	19.9	8300	0	4500
9-Sep	2500 CO IN 5% CO2	1/6 DIED	18.8	56000	0	2340
16-Sep	3500 CO IN 3.5% CO2	3/6 DIED	19.3	36000	0	3300
21-Sep	150 NO2 IN AIR	0/6 DIED	19.5	8700	140	0
28-Sep	200 NO2 IN AIR	3/6 DIED	19.4	9500	187	0
30-Sep	120 NO2 IN 5% CO2	2/6 DIED	18.6	50830	109	0
4-Oct	3500 CO & 110 NO2 IN AIR	6/6 DIED	19.7	7484	114	3550
7-Oct	2080 CO & 95 NO2 IN AIR	1/6 DIED	19.4	10570	104	2090
14-Oct	2630 CO & 111 NO2 IN AIR	5/6 DIED	19.4	11400	111	2632
18-Oct	1940 CO & 107 NO2 IN 4.5% CO2	2/6 DIED	18.8	45113	107	1940

Table 1. Exposure conditions and observed lethalities for small animal toxic gas exposure tests.

Figure Captions

1. Schematic diagram of the small animal toxic gas test facility. Gasses are metered into a stainless steel mixing chamber before being introduced into the exposure chamber. The composition of the gasses are analyzed in the outlet stream to correctly account for CO2 accumulation. Three of the six animals exposed have their ventilation rates recorded during the experiment.

2. Scheme for measuring ventilation parameters. The rats are pressed against the conical end of the holding tube by a steel plate to ensure that an air tight seal is maintained. Changes in lung volumes translate into air flows in and out of the pneumotach at the opposite end of the tube. A metal tube provides cooling of the rat's tail and a means to access the tail for blood sample measurements.

3. Variation of minute volume with CO2 concentration. Data from a subset of the experiments is shown, divided into exposures with CO, NO2, and CO + NO2. Exponential regressions are made to each data subset, assuming a common minute volume value at 0% CO2. The results show increased minute volumes with all toxic gas components, but with a lesser increase when NO2 is present.

4. Lethality correlation assuming statistically independent toxic gas pathways. Single gas results follow a logistic regression when does are corrected by minute volumes. The vertical error bars indicate the amount of CO2 in the exposure atmosphere. For combined gasses,

probability of lethalities are treated as independent events. As a result, the lethality for combined exposure is greatly underpredicted, indicating synergistic processes are at work.

5. Lethality correlation assuming fractional effective doses can be added. The lethality from combined exposures are greatly underpredicted, indicating that competition between the two processes exists.

6. Lethality correlation assuming a reduced addition of fractional effective doses. Using a formula for addition which produces less than pure addition of FED values leads to a slightly better agreement with data. Although no physiological justification for the form can be offered, it suggests the benefit of seeking a physiological correlate.

7. Lethality correlation with reduced addition in terms of FED values.

8. Lethality correlation with N-Gas value. The N-Gas model is a fractional equivalent dose model that accounts for CO2 effects on CO lethality, but not for other gasses and does not explicitly include an LC50(CO) parameter that can be adjusted for the species of interest. As a result, the model under predicts NO2 lethality when CO2 is present and underpredicts CO lethality for this rat species and exposure condition.

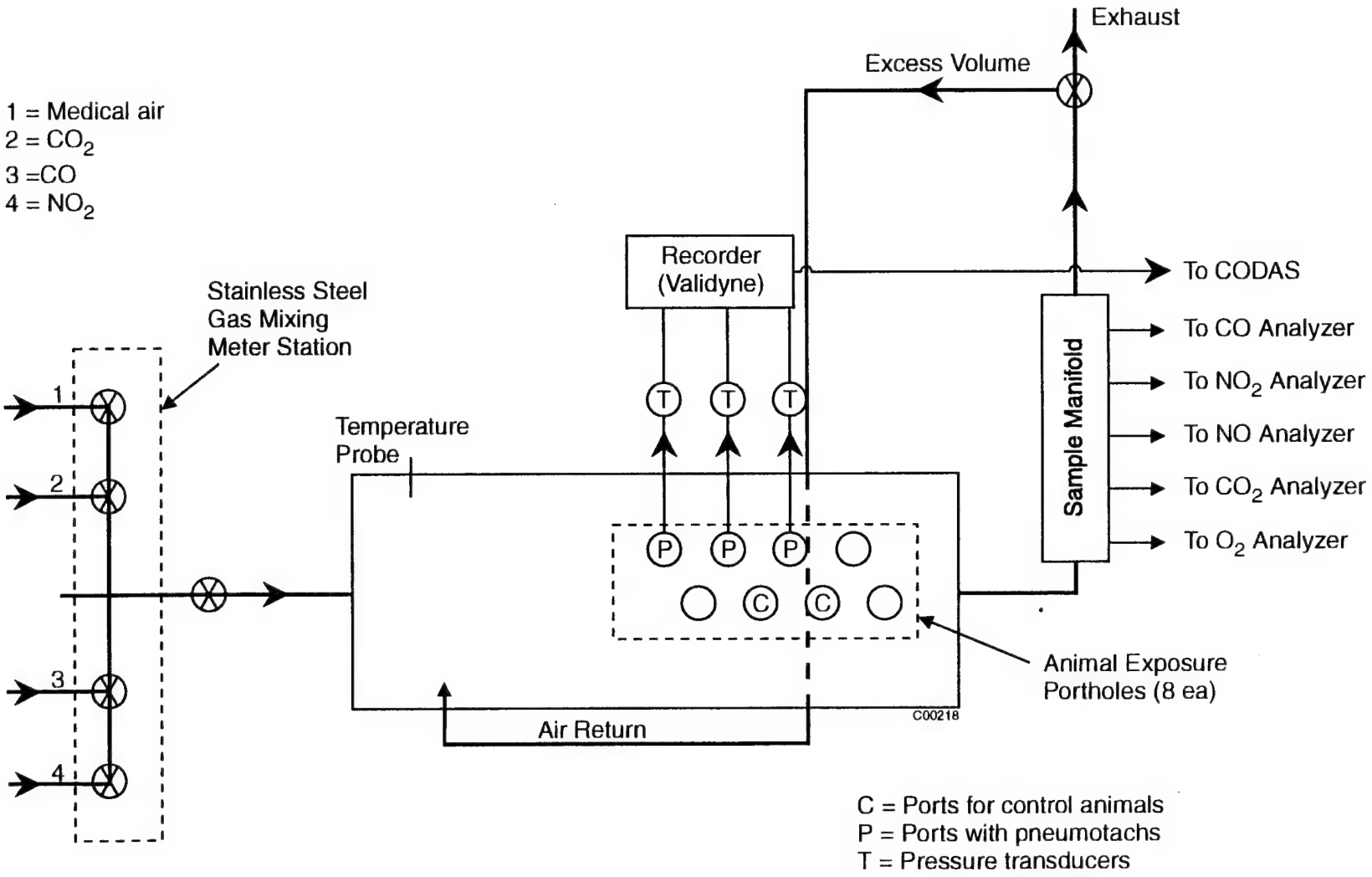

1 = Medical air
2 = CO_2
3 =CO
4 = NO_2
Stainless Steel
Gas Mixing
Meter Station
Temperature
Probe
Recorder
(Validyne)
Exhaust
Excess Volume
To CODAS
Sample Manifold
To CO Analyzer
To NO_2 Analyzer
To NO Analyzer
To CO_2 Analyzer
To O_2 Analyzer
Animal Exposure
Portholes (8 ea)
Air Return
C00218
C = Ports for control animals
P = Ports with pneumotachs
T = Pressure transducers

Figure 1.

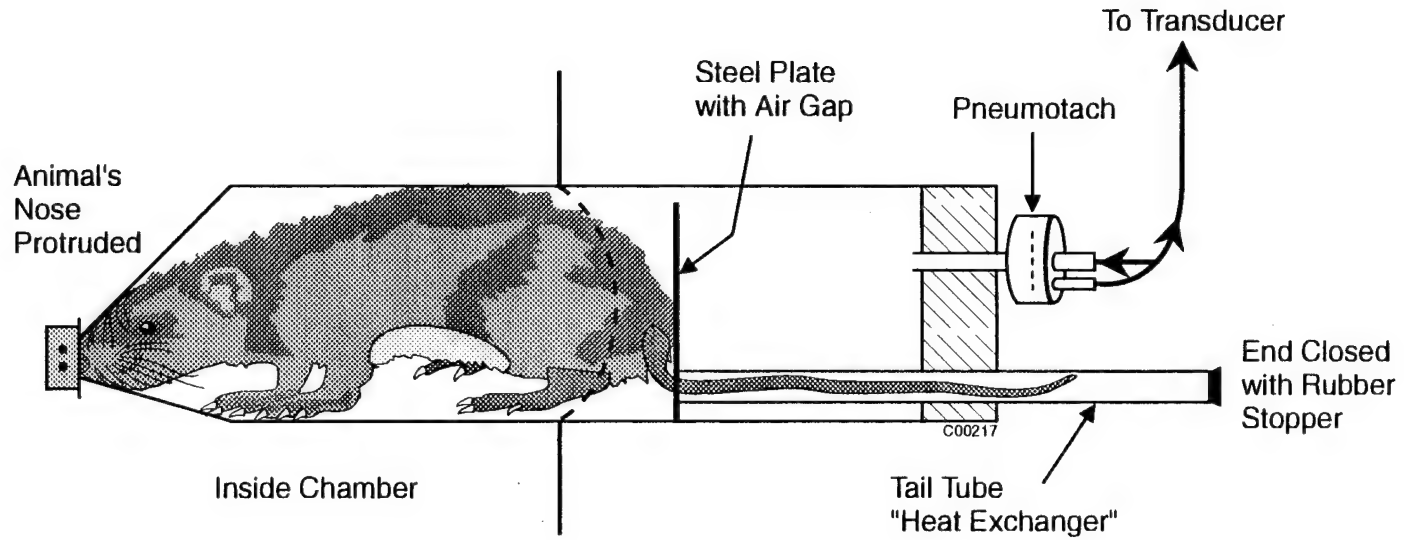

To Transducer
Steel Plate
with Air Gap
Pneumotach
Animal's
Nose
Protruded
End Closed
with Rubber
Stopper
C00217
Inside Chamber
Tail Tube
"Heat Exchanger"

Figure 2.

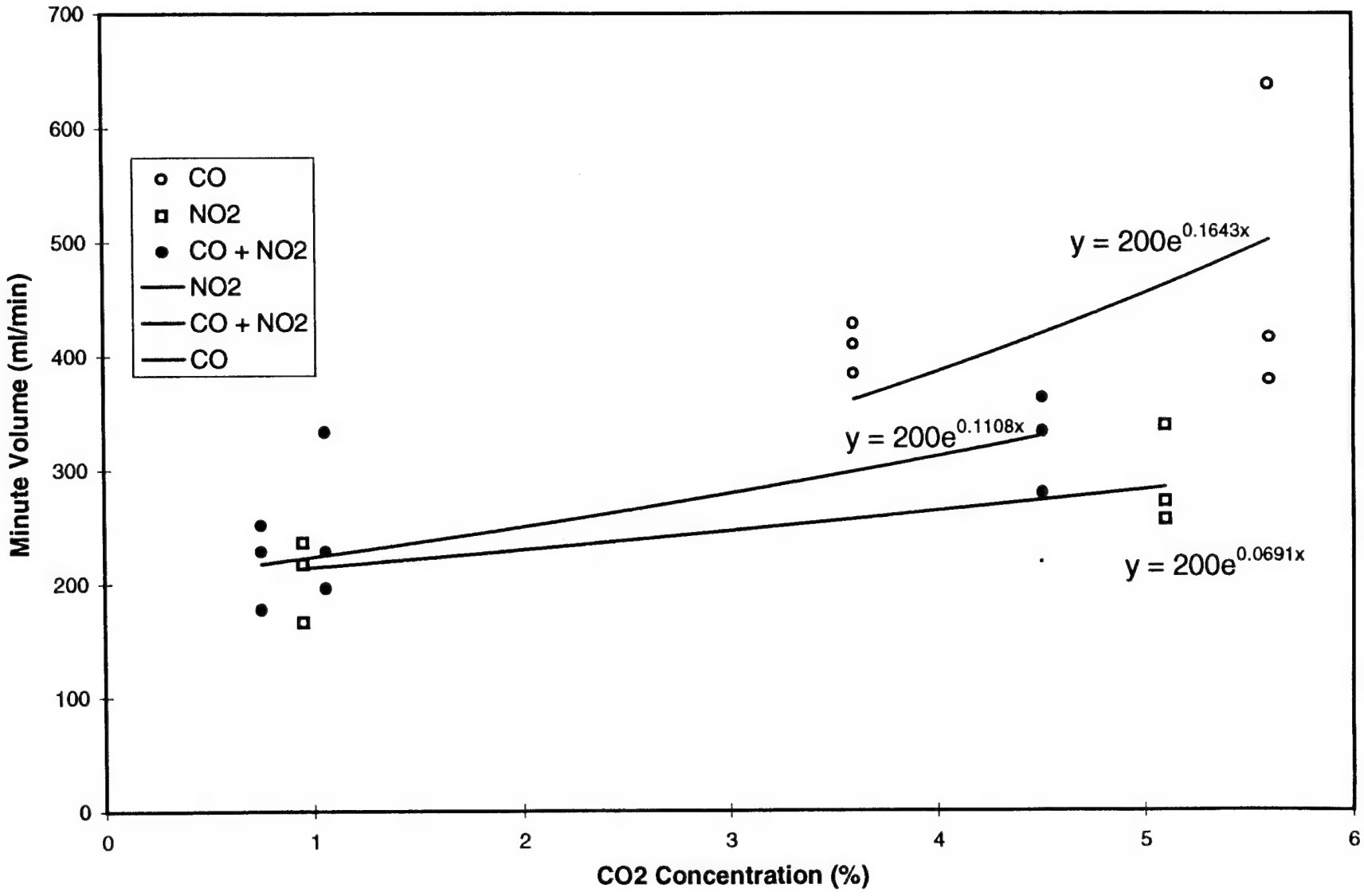

700
600
500
400
300
200
100
0
0
1
2
3
4
5
6
Minute Volume (ml/min)
CO2 Concentration (%)
CO
NO2
CO + NO2
NO2
CO + NO2
CO
y = 200e^0.1643x
y = 200e^0.1108x
y = 200e^0.0691x

Figure 3.

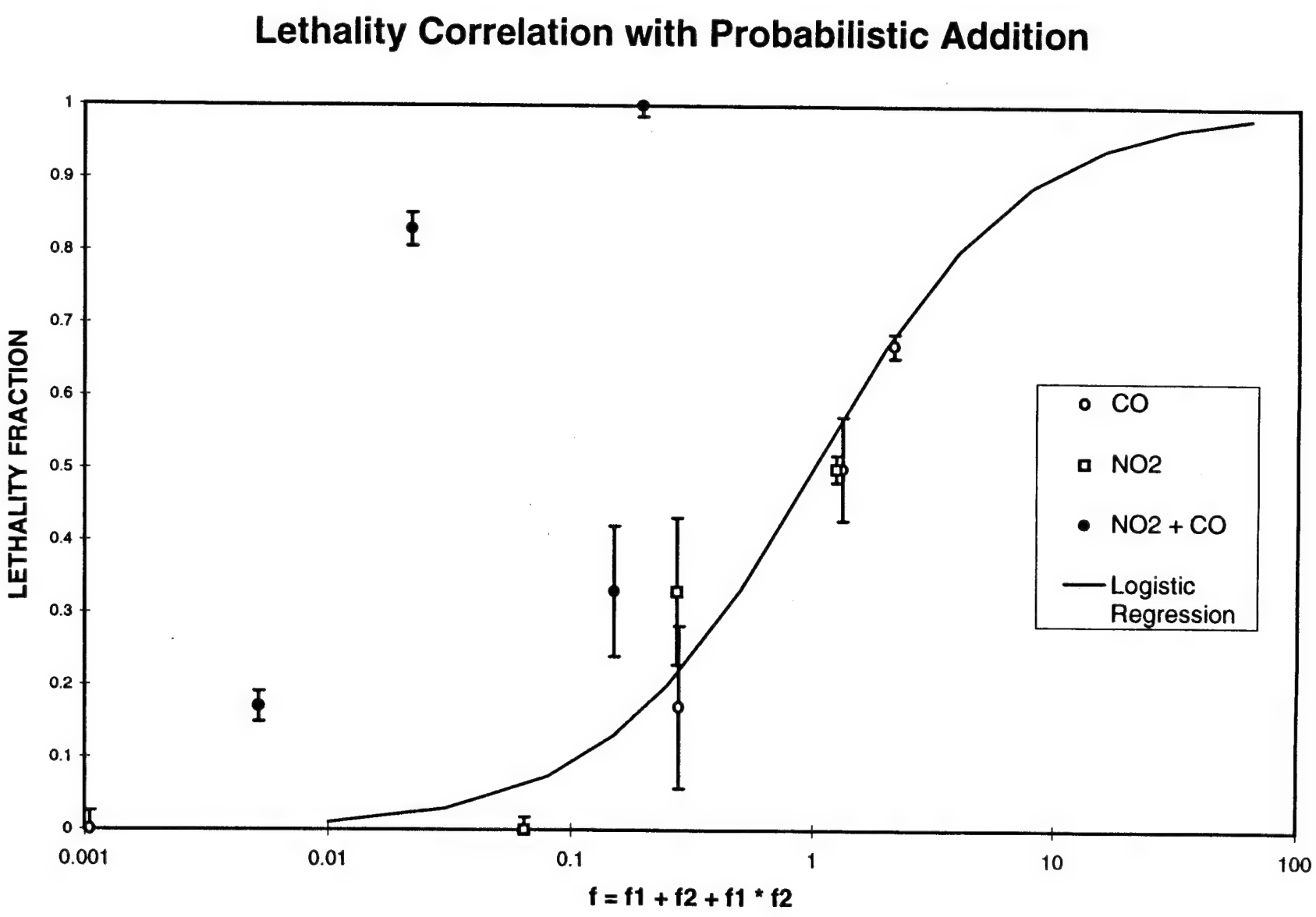

Lethality Correlation with Probabilistic Addition
LETHALITY FRACTION
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.001
0.01
0.1
1
10
100
f = f1 + f2 + f1 * f2
CO
NO2
NO2 + CO
Logistic Regression

Figure 4

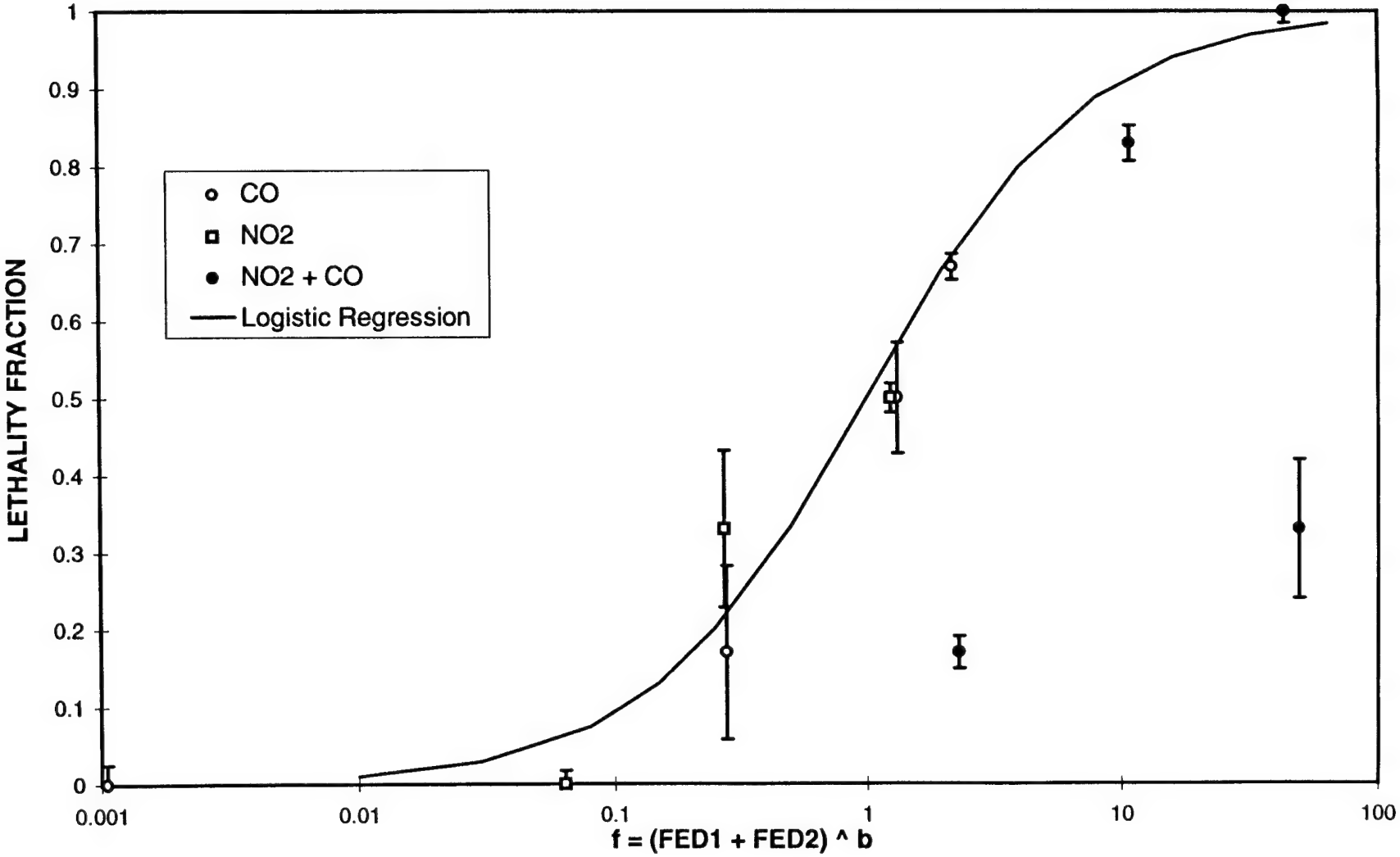

Lethality Correlation with Simple Addition
LETHALITY FRACTION
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
CO
NO2
NO2 + CO
Logistic Regression
0.001
0.01
0.1
1
10
100
f = (FED1 + FED2) ^ b

Figure 5.

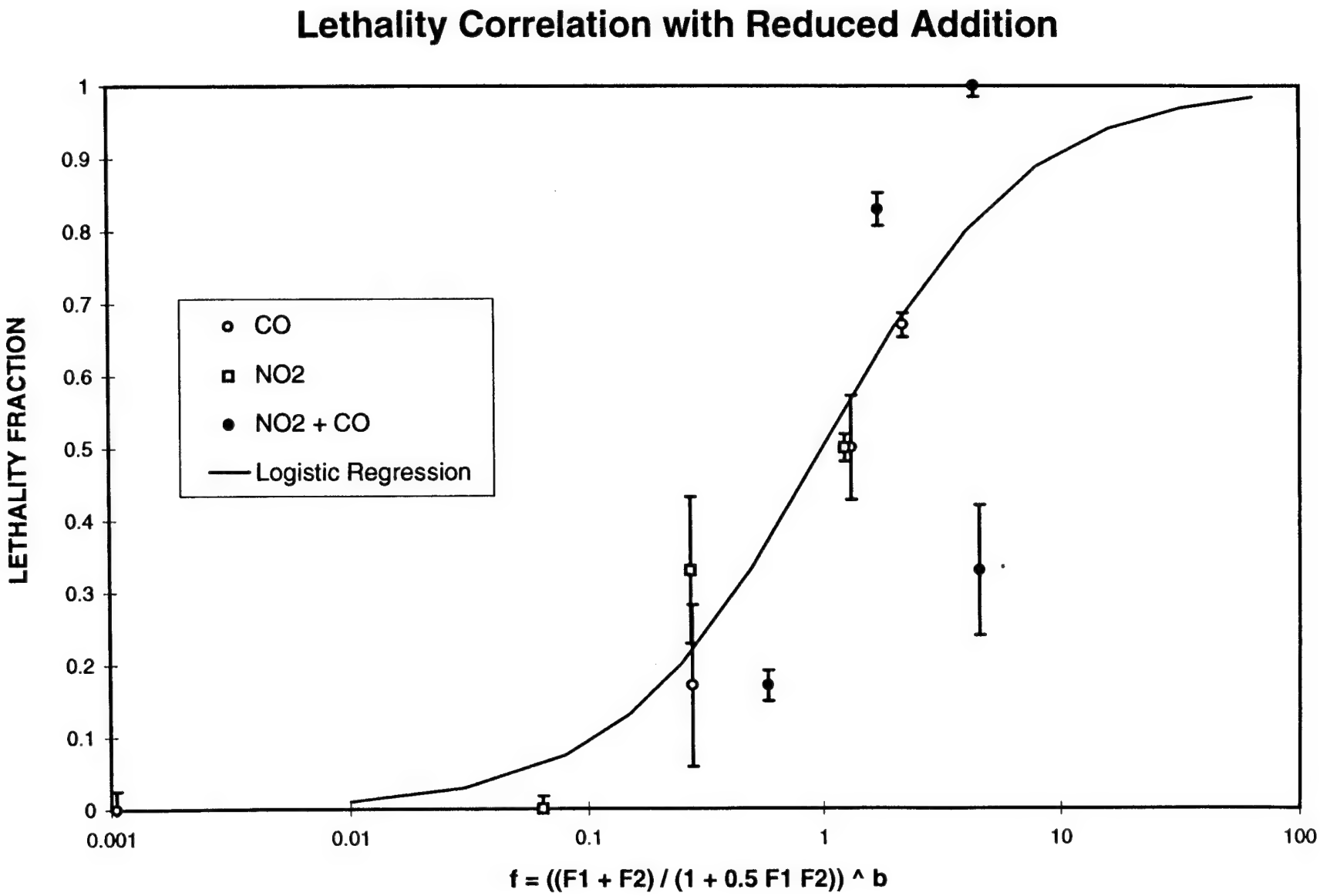

Lethality Correlation with Reduced Addition
LETHALITY FRACTION
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
CO
NO2
NO2 + CO
Logistic Regression
0.001
0.01
0.1
1
10
100
f = ((F1 + F2) / (1 + 0.5 F1 F2)) ^ b

Figure 6.

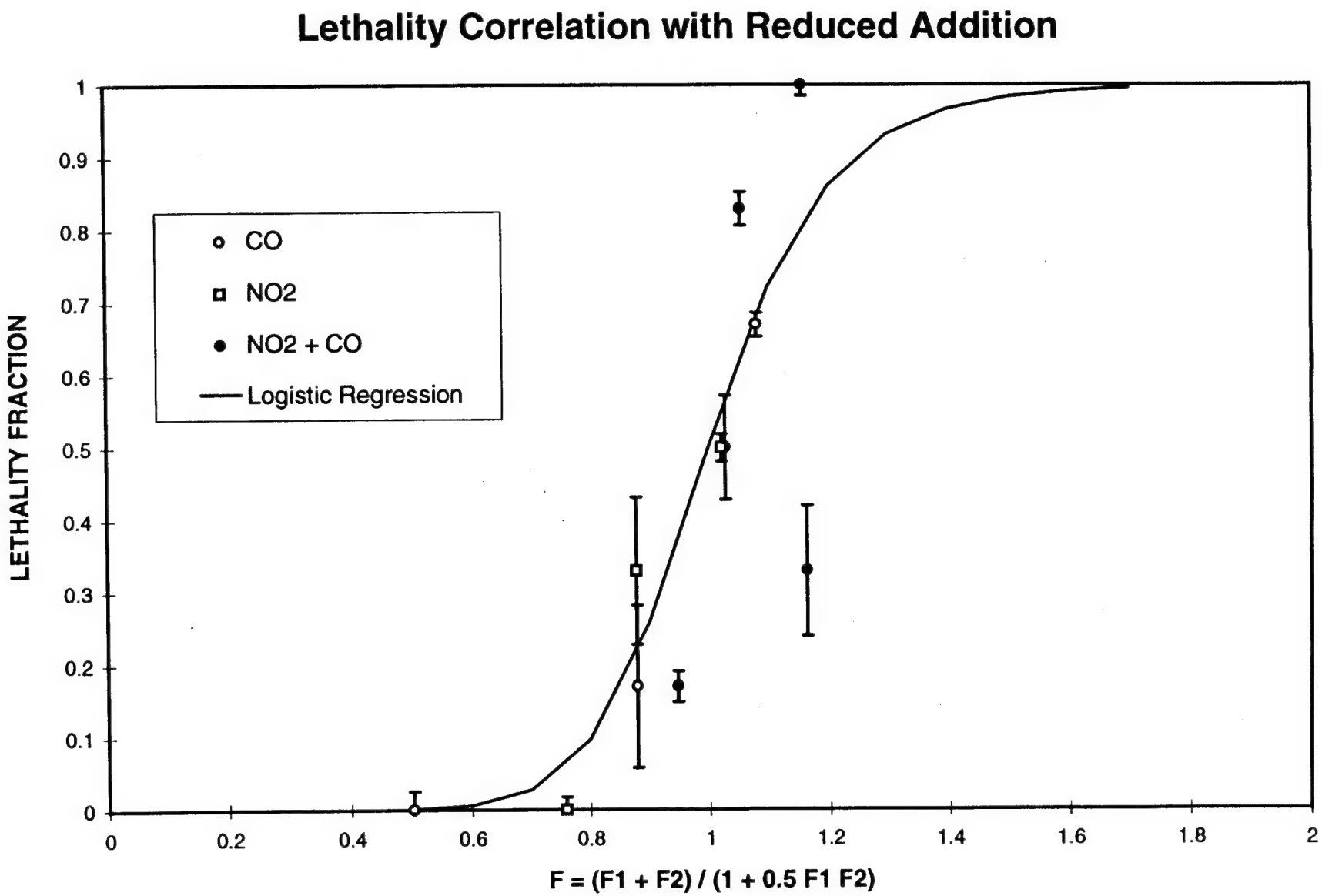

Lethality Correlation with Reduced Addition
LETHALITY FRACTION
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
CO
NO2
NO2 + CO
Logistic Regression
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
F = (F1 + F2) / (1 + 0.5 F1 F2)

Figure 7.

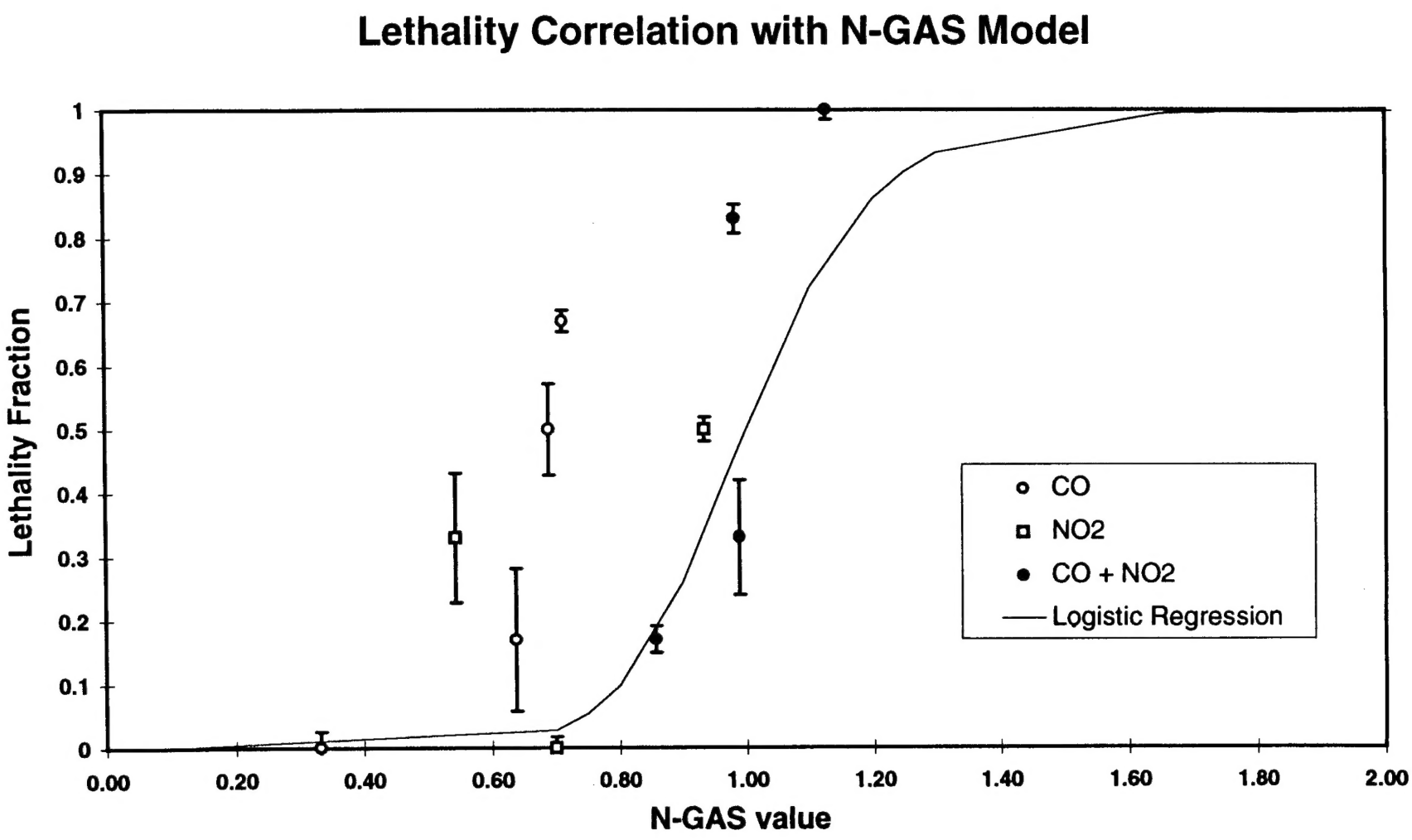
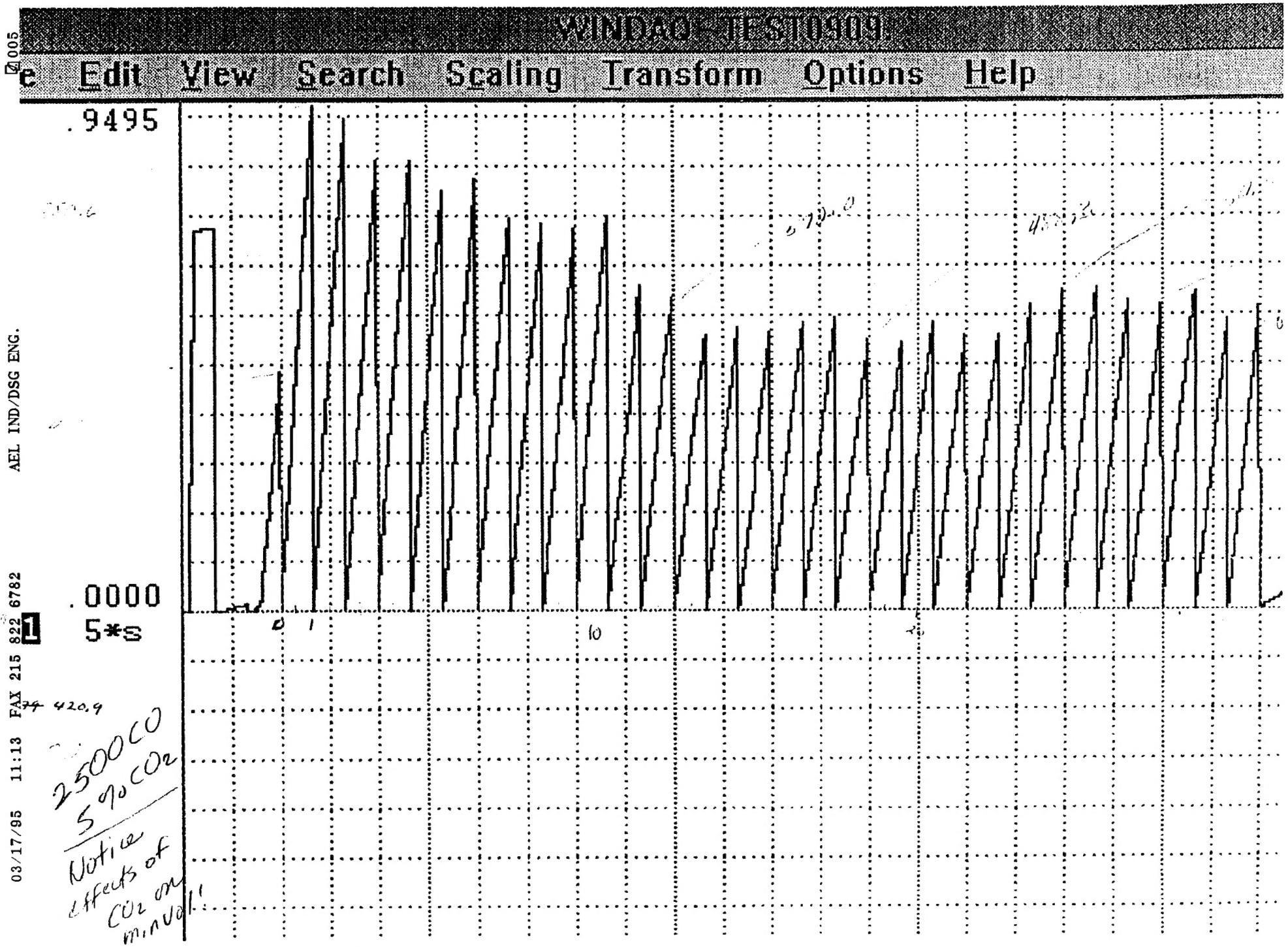
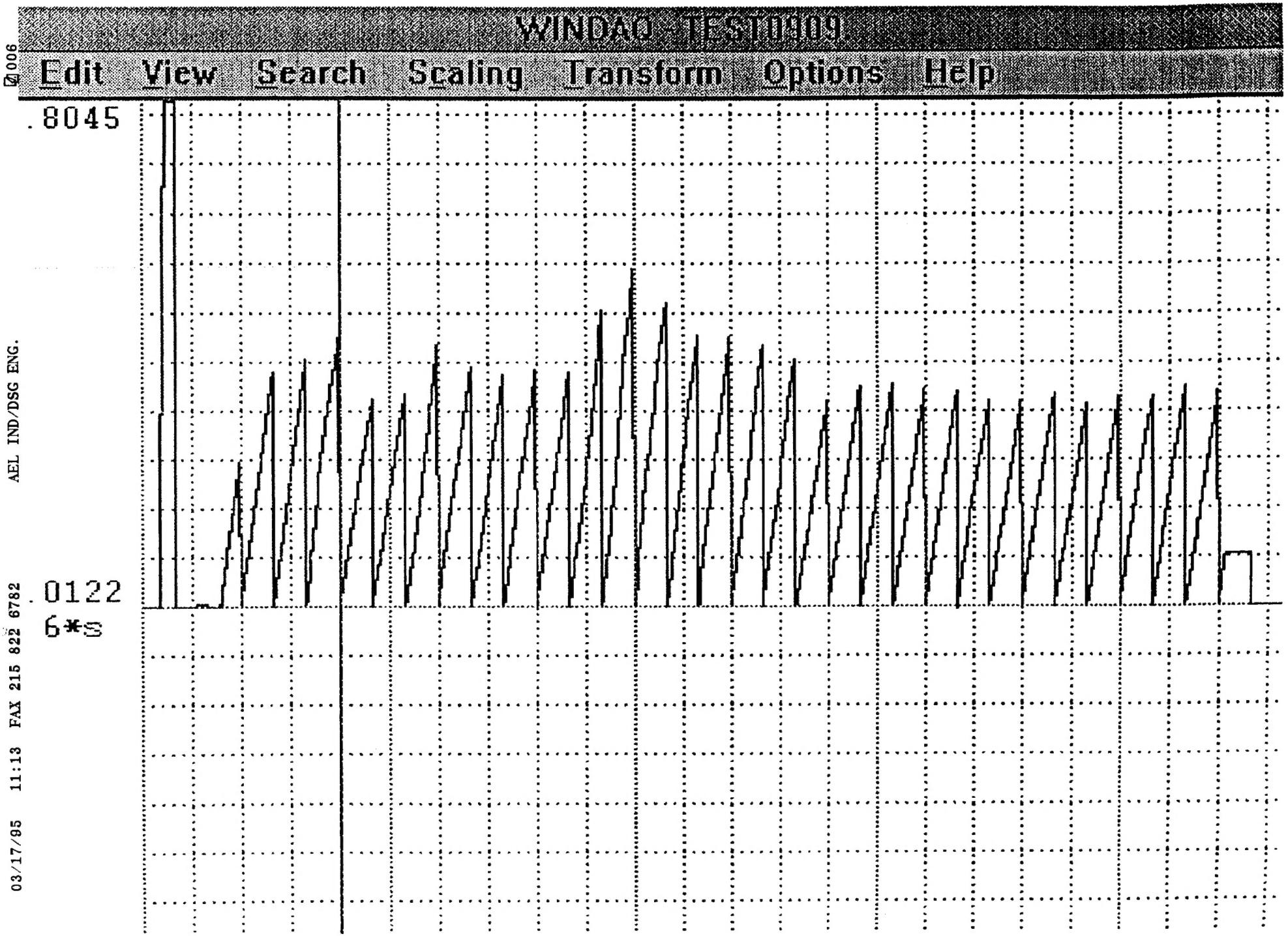

Lethality Correlation with N-GAS Model
Lethality Fraction
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80
2.00
N-GAS value
CO
NO2
CO + NO2
Logistic Regression

Figure 8.


WINDAQ - TEST0909
e Edit View Search Scaling Transform Options Help
.9495
.0000
1 5*s
0
1
10
20
420.9
2500 CO
5% CO2
Notice effects of CO2 on min vol!


WINDAQ TEST0909.
Edit View Search Scaling Transform Options Help
.8045
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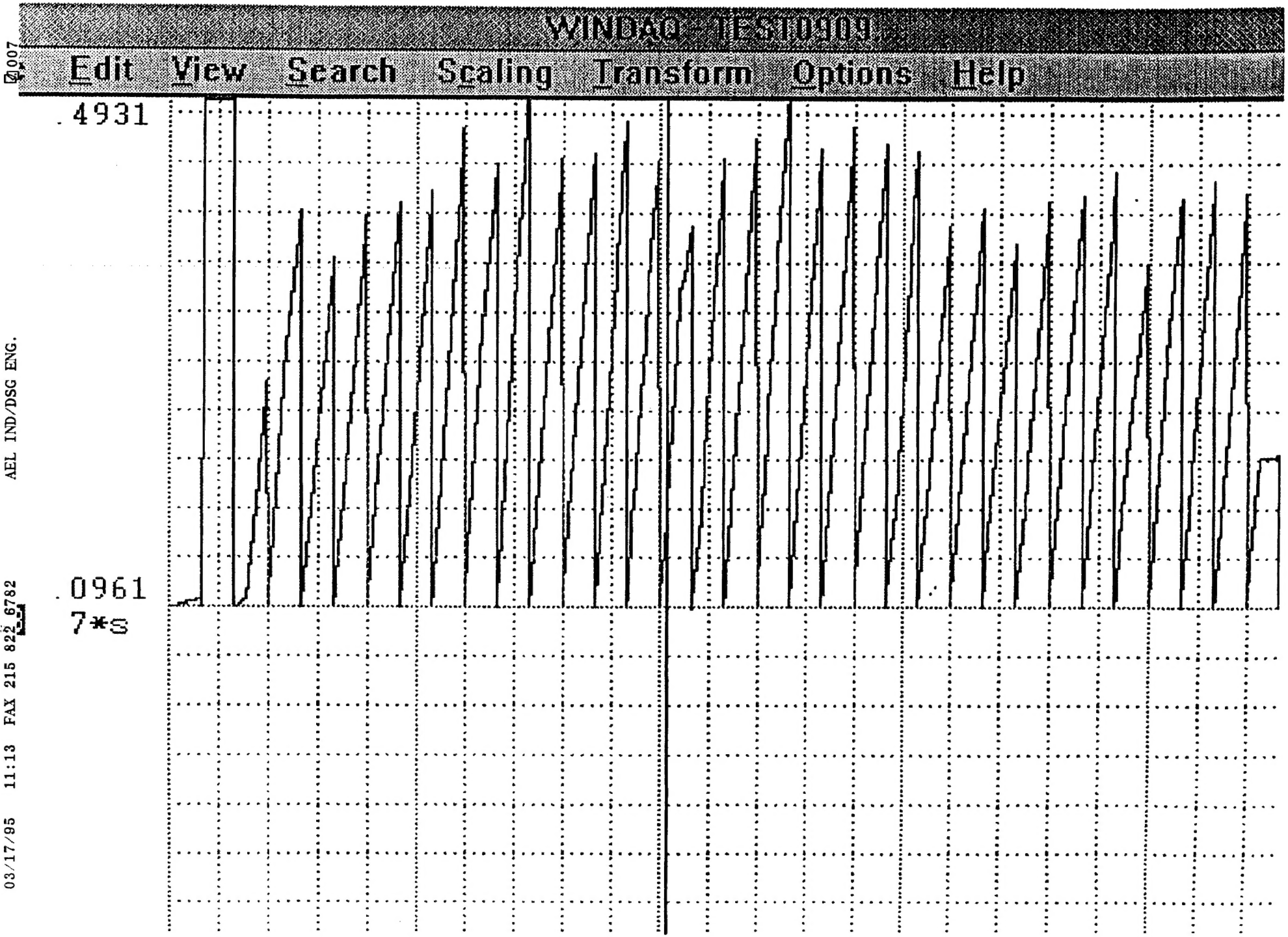
6*S
AEL IND/DSG ENG.
03/17/95 11:13 FAX 215 822 6782


WINDAQ - TEST0909
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